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IPVS2020

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2020

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IPVS2020: Memories of resilience

It had all started in Dublin (June 2016), when Brazil got the right to organize the most important congress of the international pig veterinary community: the IPVS Congress. It was a great honor, but also a tremendous responsibility. As so, we needed to start immediately with the conference planning, in other words, bringing our dreams into the real world. It took around two years with the planning, and after the IPVS2018 China, the work got even harder each day: sponsorship contacts, putting together attractive scientific and social programs.

As the Chair of the IPVS2020 Congress, I should say that our event will be known as the congress of resilience: we overcome African Swine Fever by creating a Biossecurity committee, performing a nice risk assessment, and moving the host city to Rio de Janeiro. By then, we had to rearrange our plans to adapt for the new conference venue and establish a whole new structure within a few months. And we made it! Our congress was ready to go and beautifully organized to take place from June 2nd to 5th. However, unexpectedly, the whole world faced the COVID19 pandemic in the beginning of this year. We announced the rescheduling of the IPVS2020 for November, hoping that the situation would be under control by then. Unfortunately, that was not the case. Hence, given this scenario, the IPVS2020 Local Organizing Committee, supported by the IPVS Board and the IPVS2020 sponsors, decided to postpone the IPVS Brazil to 2022. It was a tough, but was a wise decision, aiming for the safety of all involved in our event (delegates, sponsors, and organizers).

After all that was reported so far, the IPVS2020 could not just be taken away from one's mind without officially closing its whole cycle. As so, to acknowledge all contributors to the IPVS2020, we are pleased to deliver to the international pig veterinary community over 700 abstracts and the lectures, which are compiled in the IPVS2020 Proceedings. We wish to thank the reviewers for reading, scoring the abstracts and assisting with the organization of the program. We would also like to thank the IPVS Board members and all sponsors for their support, which was crucial to preserve the LOC's soundness.

With this, we completed the IPVS2020 organization cycle, but the dream is not over yet: we would like to invite you for the IPVS2022 in Rio de Janeiro.

Thank you very much for all your support, and hope that you enjoy the IPVS2020 Proceedings!

Prof. Fernanda Almeida
Chair of the IPVS2020

To the Readers

As mentioned by our president, “Resilience” or “Persistence” would represent very well the IPVS2020 by all aspects stated by her, but also considering the attempts to bring this great event to Brazil for the second time.

For the IPVS2020, the organizing committee tried to innovate and decided to offer three options of pre-conference sessions: Welfare, African Swine Fever and Reproduction, that were supposed to be held in the afternoon of the Opening Day. At the Opening Ceremony, we would have the Tom Alexander Memorial Lecture, by Dr. Peter Davies, talking about “Predicting the challenges - swine health and production, 2050?”. For the congress, we have chosen 15 subjects divided in 20 sessions and each of these sessions would have a keynote speaker to introduce the major topics. The final program would consist of 116 oral presentations, chosen among more than 800 abstract submissions. This large number of abstracts imposed a great task for the reviewers from five different continents. For all their support, we wish to thank the following reviewers for reading and scoring the abstracts:

Abelardo Silva Jr
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Satoshi Otake

For the convenience of the readers, the proceedings were compiled in only one volume, organized in sections comprised by:

I) The Tom Alexander Memorial Lecture;

II) Manuscripts from the keynote speakers from all sessions;

III) Manuscripts from the speakers of the three pre-conference sessions, and

IV) All abstracts also divided by the thematic area.

The Scientific Committee hopes that the information contained in this book can be disseminated and shared to all interested professionals in up to date scientific results that certainly will contribute to the advancement of knowledge in different areas of swine production!

Prof. Roberto Guedes
Chair of the Scientific Committee

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An event of such magnitude as the IPVS2020 Congress could not happen without the financial, professional and moral support of our sponsors.

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IPVS HISTORY

The specialization in Veterinary Medicine and the current increase in the importance of swine production have demanded the cooperation of Veterinary professionals from all over the world. This cooperation aimed the development of know-how to solve problems related to raising and reproducing this animal species.

The IPVS – International Pig Veterinary Society – was founded with this objective and with the purpose to promote, every two years, a meeting with professionals from the swine production chain to discuss the studies developed by the international scientific community.

In the last 50 years, this IPVS objective and purpose have been achieved. Since the first conference held in Cambridge/England, in 1969, the scientific and technical community had shown its interest in this international forum, where all the problems related to pig production were presented and debated. Thanks to the dedication and seriousness with which IPVS representatives have treated the issues of this community´s interest, more than 40.000 people have already had the opportunity to participate in IPVS conferences, with the presentation of more than 13.000 scientific papers. All the past editions of IPVS congresses are cited below.

HISTORICAL DATA

The interest in technical-scientific development is the main motivation to organize IPVS Congresses. Many of the debates have led to the development of procedures, which were incorporated into pig production systems, aiming to increase productivity. At each Conference, a complete review of the new advances is presented in search of more efficient solutions to face pig production challenges.

Edition	Local	Date	Chair	Abstracts	Participants
1º	Cambridge, England	23 – 28/06/69	Dr. PD Storie-Pugh	123	500
2º	Hannover, Germany	23 – 26/05/72	Dr. W Schulze	179	900
3º	Lyon, France	12 – 14/06/74	Dr. J Tournut	187	854
4º	Ames, US	22 – 26/06/76	Dr. W Brandt	374	1250
5º	Zagreb, Yuguslavia	13 – 15/06/78	Dr. O Bohm	181	450
7º	Mexico City, Mexico	26 – 31/07/82	Dr. RR Necoechea	360	1250
8º	Ghent, Belgium	27 – 31/06/84	Dr. MB Pensaert	187	854
9º	Barcelona, Spain	15 – 18/07/86	Dr. JL Garcia-Ferrero	455	1026
10º	Rio de Janeiro, Brazil	14 – 17/08/88	Dr. L Roppa	368	1233
11º	Lausanne, Switzerland	01 – 05/07/90	Dr. H Keller	528	1718
12º	The Hague, the Netherlands	17 – 20/08/92	Dr. J Verheidjen	696	2004
13º	Bangkok, Thailand	26 – 30/06/94	Dr. S Laungtongkum	538	1621
14º	Bologna, Italy	07 – 10/07/96	Dr. E Seren	648	1614
15º	Birmingham, England	05 – 09/07/98	Dr. C Glossop	839	1800
16º	Melbourne, Australia	17 – 20/09/00	Dr. R Cutler	605	1614
17º	Ames, US	02 – 05/06/02	Dr. H Harris	688	1500
18º	Hamburg, Germany	27 – 01/07/04	Dr. H Bossow	872	2455
19º	Copenhagen, Denmark	16 – 19/07/06	Dr. B Nielsen	934	2486
20º	Durban, South Africa	22 – 26/26/08	Dr. DPB Evans	918	1900
21º	Vancouver, Canada	18 – 21/07/10	Dr. E Sanford	1149	2716
22º	Jeju, Sputh Korea	10 – 13/06/12	Dr. WH Lee	–	3099
23º	Cancun, Mexico	08 – 11/06/14	Dr. A Stephano	978	2560
24º	Dublin, Ireland	07 – 10/06/16	Dr. P Kirwan	1100	3552
25º	Chongking, China	11 – 14/06/18	Dr. Y Hanchun	903	5599

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Predicting the challenges - swine health and production, 2050?

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Introduction

The founding objectives of the IPVS were to provide a forum for exchanging ideas and information concerning pig health and disease, to encourage the formation of pig veterinary societies in all countries and to foster cooperation among them. In 1969, Dr. Tom Alexander chaired the Organizing Committee of the inaugural IPVS congress in Cambridge, UK. Tom embodied and shaped the soul of the IPVS by melding an eminent scientific career with ownership of a pig farm. As a consultant, for decades he steered the application of science to pig production globally. I am honored to be given the responsibility to address this 20th IPVS congress, and to celebrate the legacy of Dr. Alexander as we collectively follow his path to pursue those goals. The biggest hurdle in tackling this task is to select a topic. Previous speakers discussed the principles of pig health assurance (Dr. Hank Harris, 2010); exploiting technology to achieve happy pigs and healthy people (Dr. Dan Tucker, 2012); accountabilities in the age of transboundary diseases (Dr. John Harding, 2014); pigs in society (Dr. Jill Thomson, 2016); and the drivers of emerging diseases (Dr. Trevor Drew, 2018).

It is not surprising that two of the last three Alexander Lectures focused on emerging diseases. The disruption of global pork markets caused by the Eurasian (and particularly Asian) epidemic of African Swine Fever (ASF) is still unfolding, and is compounded by whatever the COVID-19 (SARS-2) coronavirus may inflict upon the Chinese and global economies. The animal health, economic and trade impacts of ASF will continue to evolve, and perhaps dramatically, between the time of writing this paper and the delivery of the address in June 2020. The epidemic is already unprecedented in the scale of its impact on the global pork supply, and may be the biggest disturbance to the global protein supply of my lifetime. This gargantuan issue will dominate the IPVS congress and its theme of "Knowing the Challenges to Increase Profitability". However, over the coming decades the global swine industry will confront diverse challenges beyond ASF and other emerging infectious diseases. Many of them will be even less under our control and driven by technological advances and societal change. The following thoughts are offered to prompt discussion about which challenges are most likely to influence the prosperity of pork production into the future. As a technical profession, it is a given that pig veterinarians will be mostly engaged with challenges on the production side, supporting our clients or employers in "makin' bacon". However, profitability is a fickle mistress to pursue, and the true measure of success for the pork industry will be the extent to which it maintains or expands its share of the global protein market into the future. In this context, challenges to maintaining demand may belittle the challenges we face in achieving a more efficient and reliable supply of pork.

On the bright side, overall food demand is estimated to grow around 14% per decade. Global meat trade increased from $40.2 billion in 2000 to $113 billion in 2017 according to the IHS Markit Global Trade Atlas. Less encouraging is that growth in *per capita* meat consumption has been in steady decline for several decades, and some developed countries may have already passed 'peak meat'. In 2018, poultry overtook pork as the world's most consumed meat. The FAO projects per capita pork consumption will grow at 0.8% per annum from 2015 to 2030, versus 2.4% annual growth for poultry and growth in seafood consumption may be greater still. However, these estimates are largely projections based on prior trends, and are vulnerable to unanticipated events, disrupting technologies and societal flux. The next 30 years promise to deliver rates of change in how we live, and what we eat, at scales beyond what we have previously experienced or perhaps can even imagine.

What does challenge profitability?

An old joke attributed to Thomas Carlyle says that if you can teach a parrot to say 'Supply and Demand' you will have an economist. At an enterprise level, the link between health challenges and profitability is both self-evident and all too familiar. However, at the level of a national industry, and even more so in global markets, relationships are more complicated (Buhr et al., 1993). Aggregate supply or demand can change abruptly due to multiple factors, not least politics, and distort markets in the short to mid-term. In 2009, the Canadian government instituted a $75 million buyout plan for its pig industry - not due to poor production, but to a market crisis arising from currency exchange rates and USA meat labelling laws among other factors. Other examples from the USA include the negative effect on farm profitability resulting from the introduction of highly effective PCV2 vaccines around 2008. Improved pig survivability rapidly expanded supply leading to a prolonged period of depressed prices and producer losses. Conversely, shrinking of the pig supply due to the PED epidemic in 2014 created conditions of high profitability for many producers. Furthermore, the economic consequences for affected herds that lost large numbers of pigs was highly variable and

influenced by timing of outbreaks in relation to the aggregate supply. Producers affected early in the epidemic lost large numbers of pigs when industry profitability was marginal or negative, but had returned to full production by the peak of the epidemic when aggregate supply was depressed and profitability per pig was around $70. In contrast, herds with similar production losses, but occurring at the height of the epidemic suffered major economic losses due to inability to participate in the price boom. In the absence of expanding demand, collectively good production performance across an industry (be it local, national, or global supply) is likely to be detrimental to producer profitability and primarily benefit customers and consumers.

The current massive deficit in the global pork supply caused by ASF in Asia is delivering unusually profitable conditions for pig industries in much of the world. However, the distribution of the profit windfall from this supply gap is mostly determined by conditions in local markets. An analysis by Dr. Dennis DiPietre in December 2019 compared the impacts of the ASF induced export opportunities on industry profitability in Spain and the USA (DiPietre, 2019). In the USA, packer profitability was at record levels while producers were selling pigs at a loss. In contrast, producer profitability was extremely high in Spain, but the packing sector was struggling. The simple explanation was the balance between local production in relation to packing capacity. So even with extraordinarily favorable conditions of insufficient global supply, the economic benefits reaching producers are governed by local market conditions, and are in no way guaranteed.

Those engaged day-to-day in the business of swine production strive to manage challenges to production arising on the farm (production risk), while the vagaries of markets (market risk) are 'left to chance' or addressed with strategies such as hedging on feed and pig prices. In recent US experience, when volatility in feed and hog prices has been relatively high, the success (or otherwise) of risk management strategies has had much more influence on farm profitability than production performance. This was rarely the situation in bygone eras, when variability among farms in performance was much greater and market conditions (particularly feed prices) were more stable. Today, good production performance is necessary, but it is not sufficient to ensure economic viability, and disease remains the biggest 'wild card' disrupting herd productivity.

The paradox of progress

Much human endeavor is invested in the concept of progress – that through advances in science, technology, liberty, and democracy we can deliver a better quality of life for humankind. The concept of the 'paradox of progress' has been traced to Adam Smith in the 18th century (Heibroner, 1973) and many publications have adopted this title to highlighted paradoxes such as:

- Higher economic growth and consumerism lead to more stress as people work more and society falls behind
- The industrial and information age has created a world that richer in opportunity but is more dangerous
- As society moves forward, more problems are created
- The better things become, the worse they are perceived

Modern food systems are a poster child for the paradox of progress. The ability for average citizens to indulge their dietary or non-dietary preferences (wisely or otherwise), is a hallmark of progressive societies. In developed countries, the transformation of food procurement from hunter-gathering and subsistence agriculture (i.e., the pinnacle of eating local) to one-stop supermarkets straddling complex global supply chains is virtually complete. The vast majority of citizens play no part in producing their food, and have little understanding of it. As with electricity or running water, the facility with which consumers (particularly those of us living at high latitudes), can select from a panoply of fresh fruit and vegetables, among other foods, all year round is taken for granted - yet would be sorely missed were availability suddenly interrupted. In my view, the evolution of the food industry over the last 100 years is a paragon of progress in terms of efficiency and convenience of access to diverse dietary options, and in freeing our time for work or leisure. However, ranging from the highly technical to the sometimes hysterical, questions and criticism abound regarding the sustainability of 'food systems' into the future, particularly regarding animal agriculture (Bene 2019). Agriculture already covers over 40% of the ice-free and desert-free land area on the planet and uses about two-thirds of freshwater withdrawals (Poore and Nemechek, 2018). The sheer scope of feeding 8 to 10 billion people means that profile of the food industries of the future will, in no small part, be shaped by environmental sustainability questions and their relative efficiencies and externalities (Balmford et al., 2018).

Since the previous IPVS in Brazil in 1988, the gray-haired among us have observed laudable 'progress' in most facets of pork production. In my field of research, it is irrefutable that the safety of pork in developed countries has improved markedly in the modern era (Davies 2011). Pork is now produced at lower cost and with less environmental impact than 50 years ago, and real prices to consumers have dropped accordingly. FAO data indicate that total direct greenhouse gas emissions from US livestock have declined 11% since 1961, while production of meat more than doubled (Mitloehner, 2018). But, paradoxically, the modern pork industry, and intensive animal production generally, is widely perceived to be 'worse' than its previous (mythically ideal?) incarnations. In the USA and other developed

countries, meat industries are constantly implicated in diverse problems that are sociological (e.g., loss of small farms and impact on rural communities); environmental (e.g., odor, point source pollution, carbon footprint); sanitary (e.g., zoonotic and emerging diseases; antimicrobial use and resistance; food safety) and ethical (e.g., questioning the acceptability of animal housing conditions, traditional farming practices such as castration and tail docking; and carnivorism itself).

Constructive criticism is important in all industries to address real externalities and to guide industry practices in directions that conform to the interests of the overall society. But to be constructive, criticism must be founded on holistic analysis that balances the inevitable tradeoffs inherent in changes to, or constraints upon, industry operations. 'Career' critics of the food system in general, and intensive animal production in particular, are primed to pounce on any event that can be portrayed as industry malfeasance, and facts appear to optional (Davies, 2010.) Pressures to reform livestock production currently have considerable momentum via both legislative and commercial avenues, and in the battle to sway public opinion. Today when the vast majority of our customers have negligible experience of farming, we should expect and be sensitive to the fact that their perceptions and values regarding many aspects of animal production (e.g., housing, castration, tail docking, antibiotic use) will be different from ours. The fact that they will continue to be electronically bombarded with negative messages of our industry is also a given. So one of the biggest challenges will continue to be counteract misinformation and demonstrate that our systems and practices of pork production are consistent with contemporary values and acceptable to the bulk of normal and reasonable consumers. We also need the wisdom to realize when they may not be (Davies, 2010).

Technology– friend and foe

Progress demands change, but the familiar quote from Heraclitus (around 500 BC) that 'change is the only constant in life' is misleading. As explained by the futurist author Ray Kurzweil 'analysis of the history of technology shows change is exponential, contrary to the common intuitive linear view. So we won't experience 100 years of progress in the 21st century- it will be more like 20,000 years of progress' (Kurzweil, 2001). Our greatest challenges and opportunities lie in effectively harnessing new technologies that can revolutionize the way we do business. Dan Tucker previously highlighted the potential of some emerging biotechnologies in relation to pig health and welfare (Tucker, 2012). We have since seen that gene editing has huge potential for opening new avenues for disease control (Burkard et al., 2017). However, much regulatory and consumer terrain has to be navigated before we will enjoy the promise of these approaches (Ruan et al., 2017). More broadly, the era of 'big data' and 'blockchain' is in its formative phase but promises to transform food industries (Sylvester, 2018), and we need to keep our eyes trained on what technology may do to competition in the marketplace.

There are more than a few warning signs that technology together with societal changes could fundamentally disrupt the protein market. It may not be an overstatement that we are 'on the cusp of the deepest, fastest, most consequential disruption in food and agricultural production since the first domestication of plants and animals ten thousand years ago" (Tubb and Seba, 2019). We all should be familiar with the current intensity of investment into innovations targeting alternatives to animal protein, be they cultured animal proteins ('clean meat'?), plant based proteins and diets, and insect or algal proteins (Henchion et al., 2017). Some of the early initiatives have already experienced setbacks in the marketplace, but it is a long road, they do not all need to succeed, and we ignore them at our peril! Note that some of the biggest players in the animal protein industries are now also entering the alternative protein world. Given the deep cultural roots of eating habits, consumer behavior can be expected to lag behind technology in rate of change; however, generational shifts in food preferences are already well documented. Survey studies suggest Generation Z and Millennials are around 10% less likely to purchase meats than are older shoppers. Food choices are increasingly a statement of identity in wealthy societies, and the intersections of politics and culture with generational change and social media create very unpredictable terrain for 'technology' based and conventional proteins. Henchion et al. (2017) concisely point out that 'novel proteins require the development of new value chains, and attention to issues such as production costs, food safety, scalability and consumer acceptance. Furthermore, positive environmental impacts cannot be assumed with novel protein sources and care must be taken to ensure that comparisons between novel and existing protein sources are valid.'

Some of the more radical projections surrounding animal production are associated with the emerging technology of precision fermentation (Tubb and Seba, 2019). Briefly, precision fermentation (PF) involves the programing of microorganisms to produce specific complex organic molecules (e.g., proteins). Theoretically, this would enable decentralized and urbanized protein production, vastly reducing land requirements and transport. In 2000, producing a kilogram of protein molecules using PF cost around $1 million, but is currently of the order of $100. More telling are projections that PF produced protein will cost less than $10 per kilogram by 2025, and may be 5 to 10 times cheaper than conventional animal proteins by 2030 to 2035 (Tubb and Seba, 2019). The authors make striking predictions about the impact on livestock industries, particularly the dairy industry, together with claims of substantial nutritional and environmental benefits to society. There are vigorous debates about the promises and limitations of food systems founded on technology versus nature. Some of the wisdom put forward includes: 'techno-utopianism needs to

come with a hefty side order of the precautionary principle' and 'no industrially generated food could provide the right mix of dietary constituents essential for health'. I am agnostic about whether some or all of these predictions will come to pass. However, I am convinced that technology will have an increasing and substantial role in disrupting the conventional animal industries in the long term, and certainly well before 2050. Watch this space!

Climate change – supply vs. demand!

Ignoring climate change is something of a popular pastime, and raising it may be seen as borderline offensive in some circles. Opinions on this subject among IPVS attendees will doubtless be divided and divisive. You love Greta Thunberg or you do not. My native home of Australia is now in the midst of long-predicted extremes of drought and heat, and unprecedented destruction by fire, including substantial damage in agricultural areas. Beef producers in Australia are now importing DDGS from the USA to replace cottonseed meal in cattle diets due to cotton crop failures. The oyster harvest in Louisiana in 2019 was 50% of normal, largely because of reduced coastal salinity linked to extreme flooding in the Mississippi basin, which also reduced areas planted to corn and soybeans. Agriculture is sensitive to weather. Does anybody engaged in agriculture really believe that climate change is not a 'challenge' to be confronted by livestock industries over the next 30 years? The point here is not to debate the science and the causation of what are irrefutable increases in atmospheric CO_2 and global surface temperatures, but to consider how both the perceptions and the realities of climate change may influence pork demand and pork production over the coming decades. Bene at al. (2019) posit that extreme weather events (e.g., droughts and floods) are not drivers of food systems, although they affect them. However, they add that increases in the frequency and the intensity of extreme events at some point will become a driver 'as people, individually or collectively, will start to adapt (changing their behavior and/or technology)' leading to 'durable' changes in food systems.

An obvious issue is consumer perception of the link between animal agriculture and climate change and its potential effect on demand. Estimates of livestock's contribution to greenhouse gas (GHG) emissions have varied widely. Due to methodological deficiencies, the most influential report ('Livestock's Long Shadow' from the FAO in 2006) is now acknowledged to overestimate the livestock contribution to GHG considerably (Mitloehner, 2018). Regardless, it has had an indelible impact on societal perceptions and continues to be quoted frequently. In the USA in 2016, the major sources of GHG emissions according to the US Environmental Protection Agency were electricity production (28%), transportation (28%) and industry (22%). All of agriculture accounted for 9%, with animal agriculture contributing 3.9% (Mitloehner, 2018). A modelling study estimated that complete removal of animals from US agriculture would reduce agricultural GHG emissions by 28%, but total GHG emissions by only 2.6% (White and Hall, 2017). But can such information have any real impact on what has become 'conventional wisdom' about meat being a primary driver of climate change?

Perhaps a greater climate change concern for the swine industry will be volatility in feed prices due to more extreme and less predictable weather patterns. Greater variability in nutritional value of crops is also predicted. There appears to be little disagreement that the impact of climate change will not be uniform, and that there will be winners and losers, and there is evidence that climate changes to date have been beneficial to corn growth in the USA. This is an area of great complexity and uncertainty that is beyond the scope of this paper, but is discussed in some detail elsewhere (e.g., Myers et al., 2017).

Where will all this leave us?

In much of the developed world, meat consumption has evolved from being a luxury expense for celebratory events to an almost ubiquitous staple. Consumption of pork products is deeply embedded in many cultures and will not disappear overnight. An unanswerable question is whether the collective pressures of future challenges faced by our industry will ultimately shift the pendulum back towards pork being a luxury item versus a regular staple. The well-established associations between increased consumption of animal protein with income growth and urbanization are still evident within and among countries. It is therefore a small stretch to suggest that the evolution of income and wealth inequality globally will have some role in the positioning and perception of pork as a luxury versus a staple. Absent some biological realignment, due to feed efficiency alone pork will not win the price battle with poultry or seafood, although it will continue to compare favorably with ruminant protein. We have come so far in productivity gains that further incremental improvements are going to be increasingly hard won. Can we really do much better than maintaining farrowing rates of 90% and average litter sizes of 14 pigs without negative consequences such as elevated sow mortality and reduced piglet survivability and performance? Effective health management will become increasingly valued as variability in the more predictable facets of production is reduced. At least in some markets, there are strong indications that societal pressures will push, and niche market opportunities will pull, pork production in the direction of higher cost of production and likely lower aggregate demand. If implemented via regulation (e.g., sow housing and welfare standards; prohibitions on antibiotic use) we can expect these changes to be translated into trade barriers. The

next 30 years promises to be a much wilder ride than the last 30 years, and as always adaptability will be the key to survival.

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Antimicrobial Stewardship in pig health management

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Antimicrobial resistance (AMR) is a global concern, for both animal health and public health. Estimations of the burden of AMR predict that 10 million people will die annually in 2050 when no action is taken now. Many resistant bacteria (or resistance genes) can cross species barriers, and therefore exchange of resistant bacteria and resistance genes between humans, animals and the environment occurs frequently. Most obvious is the transfer of AMR from animals to humans with food borne pathogens such as resistant *Salmonella* spp. (e.g. *S.* Typhimurium DT104) and *Campylobacter* spp. (e.g. fluoroquinolone resistance). Regarding livestock-associated methicillin resistant *Staphylococcus aureus* (LA-MRSA), there is common sense that occupationally exposed people have a considerable chance to test positive for LA-MRSA. For transfer of other resistance markers (e.g. Extended Spectrum Beta-Lactamases) the extent of transmission between animals and humans is more difficult to quantify as this is not simply dependent on the transmission of bacteria but also dependent on the transmission of plasmids containing the resistance genes. These plasmids can change their host bacterium and transfer from for example *E. coli* type A to *E. coli* type B. The gut is considered to be an ideal place for bacteria to exchange genetic information. In the Netherlands, following the One Health approach, a consortium combined all recent ESBL-data from humans, animals and the environment. Following complex analysis techniques and taking into account genes, plasmids and the host bacteria, this study shows that about one-third of ESBL producing *E. coli* isolated from humans in the Netherlands, is of non-human origin. There is considerable human-to-human transmission but there is still a considerable influx of resistance into the human domain from other sources such as animals. It should be noted however that this study concerns only a specific type of resistance in a very specific context (a highly developed country at a time when veterinary antimicrobial use (AMU) was decreasing and human AMU already very low). Furthermore, these are estimates because transfer of AMR is very complex. Nevertheless, it is evident that actions should be undertaken to reduce AMU both in the human healthcare and in animals.

AMR is currently high on the political agenda. After the publication of the WHO-Global Action Plan and the adoption of the resolution on containment of AMR by the General Assembly of the United Nations in 2016, countries were requested to prepare a National Action Plan (NAP) with a One Health approach to combat the emergence of AMR. A One Health approach is generally considered essential for the containment of AMR, however, hardly half of the countries has this One Health component in their NAP which underlines the urge for action. Initially ‘’One Health’’ was mainly restricted to humans and animals, but more recently also the environment has been recognized as an important domain. The environment can be a reservoir of resistant microorganisms and resistance genes spilling over from humans and animals. However, the environment can also be polluted with antimicrobials (residues) via feces and urine of animals and humans that are under treatment, and from pharmaceutical factories with poor measures to prevent environmental pollution (e.g. via waste water). These residues can select for resistant organisms that can further spread of in the environment.

One of the 5 pillars of the WHO-Global Action Plan requests for the implementation of surveillance systems for AMR and AMU in all countries worldwide. Several countries have a reliable system implemented but there are clear gaps in data collection, in particular in Low and Middle Income Countries (LMICs). Therefore, there is limited information about AMU and AMR, particularly in these LMICs. Given the often unrestricted availability of antimicrobials without veterinary prescription and poor sanitation practices, especially in rapidly growing economies with intensive livestock sectors, AMR is assumed to be high, which is confirmed by data from case-studies. With the global trade of food products and travel of people, it is of high importance to develop interventions for AMU and AMR in all countries around the globe.

An important tool for risk management is the so called WHO (World Health Organization) CIA list (Critically Important Antimicrobials for Human Medicine). This list ranks antimicrobials according to their importance for human medicine. Five antimicrobials are in this list ranked as ‘highest prioritized critically important antimicrobials’ (polymyxins, 3rd/4th generation cephalosporines, glycopeptides, quinolones and macrolides/ketolides). These antimicrobials should not be structurally used in animal production, for example for the purpose of prevention or growth promotion, and only be used in individual cases after susceptibility testing has shown that there are no feasible alternatives. Over the past years, actions in European countries to curb AMU have shown to be effective with a 32.5% reduction in sales ofantimicrobials for animals between 2011 and 2017 in 25 European countries. Recently there is new European legislation introduced with the aim to further control the development of AMR in animals.

Initiatives to reduce veterinary AMU in the Netherlands already arise from 2008. In the presentation the actions undertaken to reduce veterinary AMU in the Netherlands will be presented with an emphasis on pig farming. One important intervention is the introduction of a benchmarking system for AMU at the level of individual farms and veterinarians. These actions have led to an almost 60% reduction in AMU in pig farming. New initiatives are currently aiming to reduce AMU via tailored interventions on the higher than average antimicrobial users which are identified through the benchmarking system.

Nowadays AMU/AMR is high on the political agenda of national and supranational organizations. There is however, still a considerable gap between policy and practice. Changes are urgently needed at practical level but this will only occur when there is a political will. It is therefore of utmost importance to use the currently existing political momentum to change the way we are exposing humans, animals and the environment to antimicrobials.

The challenges of *Escherichia coli* postweaning disease in pigs

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Introduction

All over the world *E. coli* is an important cause of a wide range of diseases in pigs, including postweaning diarrhea (PWD) (Fairbrother et al., 2019). Diarrhea due to *E. coli* may result in significant economic losses due to morbidity, mortality, decreased weight gain, and cost of treatment, vaccinations and feed supplements.Clinical signs fluctuate with time and regions and may range from mild diarrhea with 1.5 to 2% mortality and lower weight gain to severe diarrhea or sudden death with up to 25% mortality. PWD may be endemic or occur as outbreaks, and most commonly occurs in the first days through to several weeks after weaning and even after introduction into fattening herds. *E. coli* is the most common cause of PWD, although *Salmonella* and rotavirus may be associated with this disease and mixed infections may occur. Risk factors associated with the pigs (i.e. stress or level of immunity), and environment (biosecurity management on the farm) have an important influence on the occurrence of disease.

E. coli is a Gram-negative bacterial rod that inhabits the intestinal microflora or ecosystem of most mammalian and bird species, including the pig. Most *E. coli* are commensals, that is, they reside in the intestine but are not harmful for the host animal. Only a small proportion of isolates are pathogenic, being classified into categories or pathotypes based on the production of broad classes of virulence factors and on the mechanisms by which they cause disease.

Strains of the most important pathotype in pigs, the enterotoxigenic *E. coli* (ETEC), produce one or several of a class of toxins called enterotoxins, which act on the intestinal epithelial cells to induce the secretion of water and electrolytes into the intestinal lumen, causing the clinical signs of diarrhea. The most important enterotoxins, which define ETEC, are the heat labile toxin LT and the heat stable toxins STa and STb. ETEC must be able to adhere to and colonise the intestinal mucosa to permit the release of sufficient levels of enterotoxin to result in the development of diarrhea. This adherence is mediated by hair-like structures on the bacterial surface, called fimbriae or pili. ETEC associated with neonatal diarrhea may produce one or more of the fimbriae F4, F5, F6, and F41. The first three fimbriae are also known as K88, K99, and 987P. ETEC associated with postweaning diarrhea most commonly produce F4(K88) or F18 fimbriae.

A second pathotype found in pigs with diarrhea is known as enteropathogenic *E. coli* (EPEC). EPEC were initially associated with diarrhea in children, especially in developing countries. These bacteria cause typical attaching and effacing lesions, and possess a variant of the EPEC attaching effacing factor Eae or Intimin.

Shiga toxin producing *E. coli* (STEC) produce one or more of a family of cytotoxins which are known collectively as Shiga toxins (Stx) or verotoxins (VT). In pigs, the most important STEC are those that cause edema disease. These strains produce the toxin variant Stx2e (VT2e) and the fimbriae F18. Certain isolates produce both Stx2e and enterotoxins, as well as the fimbriaeF18. These isolates are associated more with PWD than edema disease. Such isolates are designated as ETEC/STEC hybrids.

More complete characterisation of isolates is performed in reference laboratories by serotyping, and phylogenetic analysis using such techniques as multi-locus sequence typing (MLST),pulse-field gel electrophoresis (PFGE), andmore recently by whole genome sequencing. This type of characterisationwill allow the monitoring of changing trends and the identification of new, emerging *E. coli* virulence determinants that could gain importance due to the pressure of antimicrobial therapy.

E. coli bacteria are constantly shed into the immediate environment of the pig via the feces, and contaminate the pens, farrowing crates, and floor. They can persist for long periods, possibly more than 10 weeks. *E. coli* is transmitted to pigs and other animals via contaminated feed, handlers, and drinking water, and infection occurs by the oral route. *E. coli* from pigs may also be transmitted to humans by direct contact, or ingestion of food or water contaminated following spread of manure, or ingestion of meat following contamination of carcasses at the slaughterhouse. Intestinal infections due to ETEC and edema disease STEC are often considered to be contagious, the same strain being found in several sick pigs from one pen and from one batch to another. These strains are usually only shed for a few days after infection, probably due to the development of immunity, and might not be detected at the time of appearance of clinical signs.

This paper aims to address how the pathogenic *E. coli* profile is changing in the face of recent pressures such as the overuse of antimicrobials, more intensive animal management and greater movement of animals, and the use of more sophisticated techniques for the identification and monitoring of pathogenic *E. coli.*

Detection of pathogenic *E. coli* associated with postweaning diarrhea

Rapid detection and thorough identification of pathogenic *E. coli* permit an early, accurate diagnosis of disease caused by these bacteria. This allows a timely and judicious choice of antimicrobial agent for effective treatment of affected animals and control of an outbreak. Accurate diagnosis also permits an informed decision for putting into place the most appropriate and efficacious preventive and control strategies, such as vaccination and management changes.

Currently, genotypic analysis such as polymerase chain reaction (PCR) is most commonly used to define the virotypes involved in an infection (Luppi, 2017). This test permits the detection of genes encoding for virulence factors such as toxins and adhesins. Primers recognising different genes related to toxins (STa, STb, and LT) and adhesins (F4, F5, F6, F18, F41, AIDA) for ETEC strains; attachment and effacement, such as eae; and Stx for STEC strains are readily available and can be used to perform PCR.

As *E. coli* grows rapidly and easily in routine culture conditions, a simple, sensitive, and inexpensive approach for the detection of the presence of pathogenic *E. coli* in samples is to perform PCR directly on DNA prepared from bacteria grown in an enrichment broth culture medium. This approach is used routinely at the EcL(Rhouma et al., 2017). For instance, multiplex PCR amplification may be used to detect the genes encoding for the enterotoxins of ETEC, Shiga toxins of STEC, and Eaeof EPEC, associated with diarrhea or edema disease. This approach is rapid and indicates overnight the presence of pathogenic *E. coli*, identifying the pathotype(s) involved in a particular case. However, it does not permit the identification of specific virotypes, as is possible when colonies are tested. Isolates from pathotype-positive cases may then be virotyped by PCR. Identification of the causative agent of disease will permit a more appropriate choice of isolate(s) for antimicrobial resistance testing. In many laboratories, hemolytic isolates are selected for further virotyping.

In a particular case, ETEC colonies positive for one of the adhesins F4, F5, F6, F41, or F18, or STEC positive for F18, when present, could be considered the causative agent of the presenting diarrhea or edema disease, as these virotypes are rarely present in the intestinal microflora of normal pigs. On the other hand, EPEC or ETEC positive for AIDA, when present, are considered as opportunistic agents of diarrhea, as strains of these virotypes have been demonstrated to cause diarrhea in pigs in challenge experiments but are also present in the intestinal microflora of normal pigs. Similarly, ETEC or STEC possessing no known adhesins, when present, would be considered as possible agents of diarrhea or edema disease, as strains of these virotypes have not yet been demonstrated to cause disease in pigs, and are also found in the intestinal microbiota of normal pigs.

Recent data on the virulence gene profile of pathogenic *E. coli* associated with postweaning diarrhea in various countries

An overview of recent published data(Table 1); Luppi et al. (2016); Yang et al. (2019); Bessone et al. (2017); Van Breda et al. (2017); Do et al. (2019) and of data reported from our laboratory (EcL)on the presence of virulence genes in *E. coli* isolated from cases of postweaning diarrhea suggests that there are some differences between countries in the prevalence of the different pathotypes. Care should be taken in interpretation of these results due to differences between studies in the methods for detection of positive isolates.Also,the combinations of virulence genes found in individual isolates were not always reported for each study.

The proportion of Stx2e-positive isolates was higher in certain countries, such as China and Korea, suggesting a higher prevalence of STEC in those countries (Table 1). Also, the data show that the relative importance of F4-positive and F18-positive isolates varies greatly from one country to another.In addition, Luppi et al. (2016) found a variation in relative importance of F4-positive and F18-positive isolates between the European countries in their study. In contrast to the situation in most other countries, F4-positive isolates are far more prevalent than F18-positive isolates in Québec, Canada.

Table 2 summarizes the pathovirotypes and virotypes most commonly observed, when reported, in the above and other recent studies for which the virulence gene combinations of isolates were reported Garcia-Menino et al. (2018); Mohlatlole et al. (2013); Brand et al. (2017). At the EcL, we refer to the combination of pathotype and fimbrial adhesin as the «pathovirotype», and use this designation in reporting results to veterinarians. This provides relevant information regarding the choice of prevention measures that may be used, e.g. a vaccine specific for a fimbrial adhesion such as F4 or F18, without the complication of details on enterotoxins, which are not necessary for such decisions.

The prevalence of F4- and F18-positive isolates in the Chinese study was very low(Yang et al., 2019) whereas the prevalence of LT-positive isolates was much higher, suggesting a high prevalence of ETEC negative for both F4 and F18 (Table 1). Similarly, we observed a higher prevalence of LT-positive isolates at the EcL. In addition, we often observe samples in which ETEC isolates negative for F4 and F18 are found alone, or in combination with ETEC:F4 or ETEC:F18 positive isolates (Table 3). Enriched primary cultures from these samples from pigs with PWD are often positive for one or more of the enterotoxins but negative for F4 and F18. In contrast, both enriched cultures and isolates from samples from healthy pigs on 12 different farms (Table 4) demonstrated a very low prevalence of virulence genes.

These results suggest the presence of previously unknown pathogenic isolates or secondary pathogens/opportunists that contribute to the development of diarrhea in certain circumstances such as co-infections and that should be taken into consideration when looking at prevention measures.

Table 1. Prevalence of virulence genes in pathogenic *E. coli* from pigs with postweaning diarrhea in different countries.

Country	Year of sampling	No of isolates examined	Proportion of isolates (%):							Reference
			LT	STa	STb	Stx2e	F4	F18	Eae	
China	2014-2016	455	62.8	15.0	33.2	21.5	2.3	3.3	9.0	Yang et al. (2019)
Argentina	2017		29.2	16.7	25	2.1	33.3	39.6	16.7	Bessone et al. (2017)
Australia	2013-2014	70	44.3	28.6	51.4	2.9	47.1	30	ND	Van Breda et al. (2017)
Korea	2008-2016	362	33.4	33.4	45	26	20.2	43.1	ND	Do; Byun and Lee (2019)
Italy, France, Germany, Belgium, Netherlands	2012-2014	339	31.9	38.1	59.1	9.7	45.1	33.9	ND	Luppi et al. (2016)
Canada (Quebec)	2014-2018	940	45.0	36.1	73.6	8.4	41.7	11.5	13.0	Fairbrother, EcL, data not published

Table 2. Pathotypes, pathovirotypes, and virotypes of *E. coli* isolates commonly observed in postweaning diarrhea in pigs.

Pathotype	Pathovirotype	Most common virotypes	Most common STs, when tested
ETEC	ETEC:F4	LT:STb:F4	10, 90, 100
		LT:STa:STb:F4	100
		LT:STb:F4:F6	
		STa:STb:F4	772
	ETEC:F18	STa:STb:F18	10, 5786,
		LT:STa:STb:F18	42, 847
		STa:F18	
		LT:STb:F18	1266
	ETEC:F4:F18	LT:ST:STb:F4:F18	
	ETEC	STb	
		STa:STb	
		STb:AIDA	
ETEC/STEC	ETEC/STEC:F18	STa:STb:Stx2e:F18	10
		LT:STb:Stx2e:F18	
		LT:STa:Stx2e:F18	
		LT:STa:STb:Stx2e:F18	88
STEC	STEC:F18	Stx2e:F18	10
	STEC	Stx2e	
EPEC	EPEC	Eae	10, 29, 48,
ETEC/STEC/EPEC	ETEC/STEC/EPEC:F4	LT: Stx2e:Eae:F18	4214
ETEC/EPEC	ETEC/EPEC:F18	LT: STb:Eae:F18	

Table 3. Common virulence gene profiles in clinical cases with intestinal problems in pigs at EcL.

Enriched sample virulence gene profile	Isolate virulence gene profiles	Pathovirotype.s	Comments
LT, STa, STb, F4	LT:STa:STb:F4	ETEC:F4	Causative agent
	LT:STa:STb:F4	ETEC:F4	Causative agent
	LT:STb, STa:STb	+ ETEC co-infection	Possible contributing agent
	STa:STb, LT:STb,	ETEC	Possible contributing agent
	STb	ETEC	Possibly in co-infection with ETEC:F4
LT, STb, F18	LT:STb:F18	ETEC:F18	Causative agent
Stx2, F18	Stx2:F18	STEC:F18	Causative agent
LT, STb, STa, Stx2	Stx2	STEC	Possible agent
Eae, STa, STb	Eae	EPEC	Opportunistic
STb	STb:AIDA	ETEC:AIDA	Opportunistic

Table 4. Virulence gene profiles in isolates from 34 samples from healthy pigs on 12 different farms

Isolate virulence gene profile	% samples with isolate showing profile
Negative	35
Eae	35
STb	15
STb + Eae	9
LT:STb:F18	3
F18	3

Emergence of clonal clusters of antimicrobial resistant pathogenic *E. coli* in postweaning pigs

In 2014, a new ETEC:F4 virotype, LT:STb:STa:F4, appeared and became predominant in cases of diarrhea in pigs in Quebec province (Figure 1) (Fairbrother, data not published). Up to 2014, the predominant virotype was LT:STb:F4. Isolates were nonsusceptible to enrofloxacin and have been becoming increasingly multidrug resistant (Figure 2). PFGE analysis revealed the presence of a clonal cluster that emerged in 2015 and has subsequently spread throughout the province of Quebec. The prevalence of cases associated with this cluster appears to have decreased more recently. From 2016, another new ETEC:F4 virotype, STb:STa:F4, has appeared and continues to become more prevalent (Figure 2, «other ETEC:F4»). We will continue to monitor isolates of this virotype in order to determine if a new multidrug resistant pathogenic clonal cluster is emerging.

Similarly, Kusumoto et al. (2016) described the emergence in 2003-2005 of an ETEC/STEC clonal cluster based on PFGE analysis. Isolates belonged to ST88 and to virotypes LT:STa:STb:Stx2e:F18, were predominantly fluoroquinolone-resistant, and demonstrated a high level of MDR.

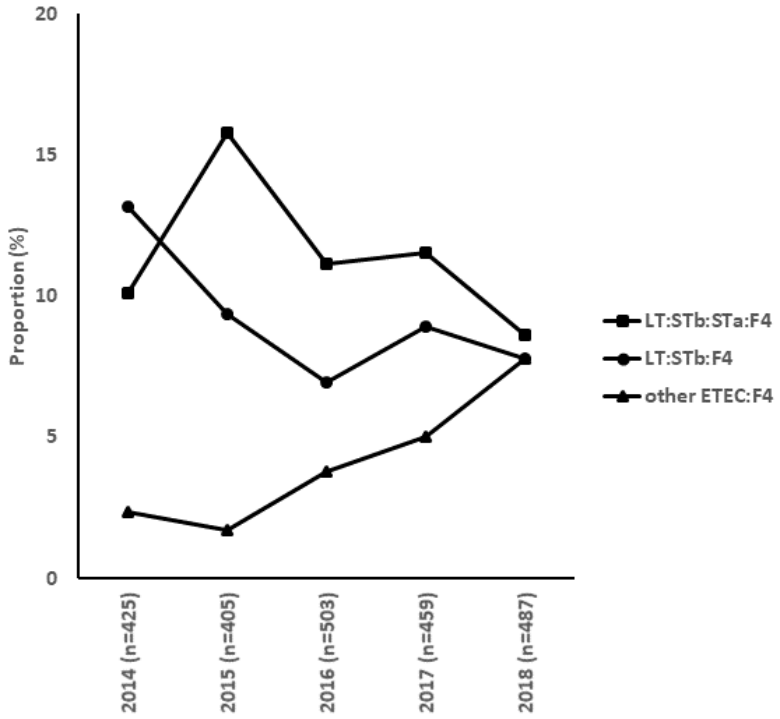

Figure 1. Proportion of cases positive for the various ETEC:F4 virotypes in Quebec, Canada, by year.

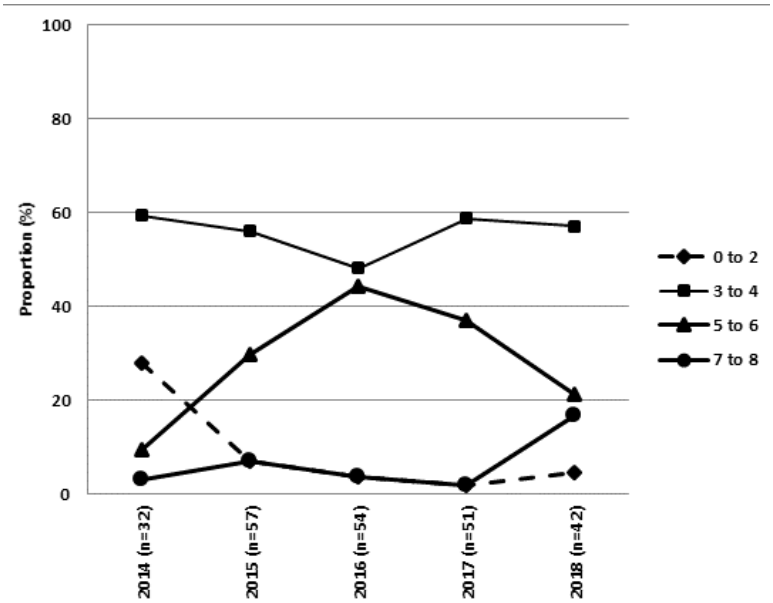

Figure 2. Multidrug resistance of LT:STb:STa:F4 isolates from diseased pigs in Quebec, Canada, by year. Isolates were tested for resistance to 8 classes of antimicrobials commonly used for treatment of bacterial infections in pigs.

Prevalent pig *E. coli*clonal lineages may have adaptive advantages due to antimicrobial resistance

As shown in Table 2, many pig pathogenic *E. coli* of the various pathotypes belong to the clonal lineage ST10. Brilhante et al. (2019), using whole genome sequencing, recently showed that the ETEC and STEC virulence genes of a hybrid ETEC/STEC:F18 ST10 strain were carried on a single plasmid, suggesting a key role for plasmids in the emergence of hybrid pathotypes. Multiple resistance genes were carried on two conjugative plasmids on this same strain. Garcia-Menino et al. (2019), also using whole genome sequencing, identified hybrid ETEC/STEC:F18, ETEC:F18, and ETEC:F4 strains of clonal lineage ST10 that possessed chromosomal mutations both for fluoroquinolone and colistin resistance, as well as plasmid-located *mcr*genes conferring colistin resistance and resistance genes for other antimicrobials.

Conclusions

It appears that persistent, multidrug resistant, pathogenic *E. coli* clones associated with postweaning diarrhea in pigs are now emerging more frequently. The additive effect of the presence of the different antimicrobial resistance genes or mutations, often both chromosomal and plasmid-located, could be conferring adaptive advantages to clonal lineages such as ST10 and driving an increased virulence andability to emerge, spread, and persist, and allowing these lineages to predominate. Tools such as whole genome sequencing and associated databases such as that for MLST will facilitate the worldwide monitoring of the emergence of new clonal lineages.

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Struggling to control *Streptococcus suis* disease in the context of antibiotic reduction

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Introduction

Streptococcus suis (*S. suis*) is a major porcine pathogen responsible for important economic losses to the swine industry. In fact, it is one of the main causes of bacterial death in post-weaned piglets, from 5 to 10 weeks of age. According to the Canadian Swine Health Information Network, *S. suis*-related diseases are the most common infectious problem reported in Canadian swine farms. In addition, the Monitoring and Analysis Working Group from the Swine Health Information Center (SHIC) reviewed and established final rankings for what is now the Swine Bacteria Disease Matrix. As stated on its website, *S. suis* leads the list as the most important bacterial swine pathogen (https://www.swinehealth.org/swine-bacterial-disease-matrix/). Clinical features of these infections in pigs are meningitis, arthritis, endocarditis, polyserositis and septicemia with sudden death. The implication of *S. suis* as a primary respiratory pathogen has been seriously questioned, and it is now considered as a secondary agent of pneumonia. Outbreaks of *S. suis* disease result in decreased performance and increased mortality, which have a significant economic impact.

It is also a zoonotic agent and the infection in humans has attracted a high level of attention in last years, with deadly outbreaks reported in Asian countries, such as China and Thailand. There are at least 35 serotypes, based on capsular polysaccharide (CPS) antigens or CPS-related genes, although some of them have been proposed as being part of different bacterial species. Indeed, serotypes 20, 22 and 26 have been re-classified as *Streptococcus parasuis*, serotypes 32 and 34 as *Streptococcus orisratti* and serotype 33 as *Streptococcus ruminantium*. Previously described *S. suis* serotypes 20, 22, 26, 32 and 34 are still recovered from diseased pigs and many laboratories do still identify such isolates as *S. suis*. Serotype 33 reference strain was originally isolated from an ill lamb (arthritis). So far, there is no single strain (confirmed by PCR) of this serotype recovered from swine: the few field strain reported in the past were identified by coagglutination (serological assay), a technique that presents many cross-reaction. When tested by PCR, these field strains were confirmed as being untypable or autoagglutinating. On the other hand, *Streptococcus ruminantium* (some of them detected as *S. suis* serotype 33 and others not) are frequently recovered from diseased ruminants (bovine and ovine) suffering from respiratory disease, abscess, arthritis, mastitis and other types of infections. *S. ruminantium* (and previous *S. suis* serotype 33) should not be considered as a primary porcine pathogen anymore.

Among the serotypes described, type 2 is the most virulent and frequently isolated from both diseased pigs and humans. Serotype 9 is another important serotype involved in swine diseases However, this is the reality of Europe, which may be different in America, especially Canada and USA. Phenotypically and genotypically different strains of *S. suis* serotype 2 (with different degree of virulence) have been isolated in different parts of the world. *S. suis* strains have also been analyzed and classified into clonal complexes (CC) composed by different sequence types (ST) when analyzed by multilocus sequence typing and, more recently, by whole genome sequencing, confirming the genetic heterogeneity within the *S. suis* species. Finally, untypable strains are also sometimes isolated from diseased animals and their possible virulence capacity should not be disregarded.

Pigs may acquire *S. suis* from the sows (during farrowing and also through oral/nasal contact) and through piglet-to-piglet transmission. Bacteria are localized in the tonsils, but they are also present in saliva: most pigs are carrier animals, harboring mostly low virulent strains. In the presence of virulent strains, some carrier piglets will eventually develop septicemia, meningitis and/or arthritis due to dissemination of *S. suis*, when maternal antibodies decline (between 5 and 9 weeks of age). The incidence of the disease is usually kept under 5% in the field. However, in the absence of prophylactic, metaphylactic and/or curative antibiotic treatments, mortality may reach 20%. Indeed, a significant increase of *S. suis*-related disease in post-weaned piglets has been observed in the last years, mainly associated to the reduction in the use of antibiotics. Although studies on *S. suis* have been significantly increased in the last 15 years, there are still many unresolved questions.

S. suis invades through the respiratory tract, gastrointestinal tract or both?

An overview of the pathogenesis of the infection can be observed in Figure 1.

It has always been accepted (and demonstrated) that *S. suis* enters through the respiratory tract. Bacteria are then located at tonsils and remain there, being *S. suis* a normal inhabitant of the upper respiratory tract. Under stressful

conditions and in the absence of antibodies (post-weaned piglets), potentially virulent strains invade the bloodstream (by still unconfirmed mechanisms) and induce bacteremia, septicemia and clinical signs (depending the colonized organs), through the induction of high levels of pro-inflammatory mediators. Sometimes, concentration of such mediators is so high that sudden death occurs and animals are simply found dead.

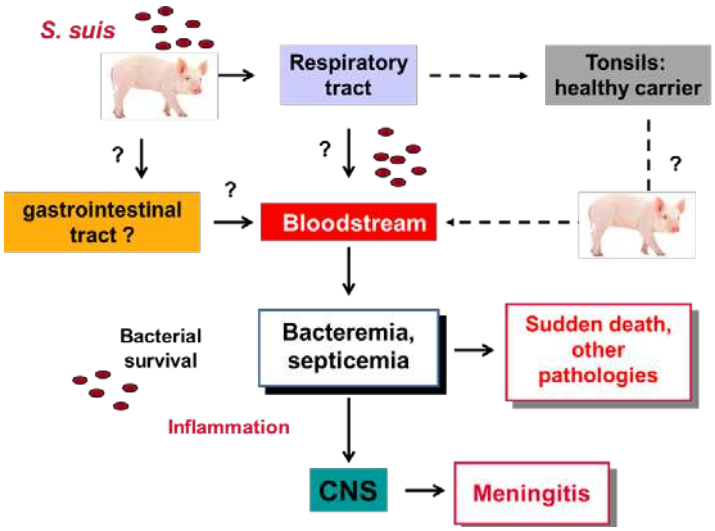

Figure 1: Proposed pathogenesis of the infection caused by *Streptococcus suis*

More recently, a new route of entry has been proposed: the oral route, as it happens very often in humans (South-East Asia). However, this still needs to be confirmed. Studies reproducing disease mimicking this route of infection (for example: intestinal translocation) used either direct inoculation of high concentration of virulent *S. suis* in the jejunum or high concentration of bacteria included inside acid-resistant capsules and then given orally to pigs. Even under these circumstances, a very low number of infected animals developed disease. When post-weaned animals were challenged through the oral route (without acid-resistant capsules), animals remained healthy. So far, many studies showing presence of *S. suis* in the intestine used DNA detection, so it is not easy to differentiate between live and dead bacteria. In addition, many of these studies included the use of genes that are not able to differentiate between *S. suis* and *S. suis*-like microorganisms. However, other studies isolated *S. suis* from feces. Indeed, in some cases of septicemia, animals may present diarrhea and *S. suis* (the strain responsible for the disease) can be isolated from feces. However, it is not clear if the simply presence of virulent strains in the intestine of clinically healthy pigs is enough to induce disease. It is important to note that stress due to weaning and feed changes may significantly modify the intestinal environment causing stress to animals, which may be much susceptible to develop disease. Usually, first animals to develop disease are those in great shape and the biggest ones. Poor adaptation to solid feed may be even more important in such animals. Stress may induce invasion of *S. suis* into the bloodstream, but this may also happen from tonsils. More studies about possible pathogenesis of the infection through the gastrointestinal tract are needed before proposing new food additives to control *S. suis* infections (see below).

What is the definition of virulent strains of *S. suis*?

The majority of porcine *S. suis* infections are caused by strains belonging to a relatively small number of serotypes. Although the distribution of serotypes from clinical cases differs depending on the geographic location, serotype 2 strains are responsible for the majority of cases in both swine and humans worldwide, and thus this serotype has been historically considered the most frequent and virulent type. However, this is true for Europe and Asia. In addition, serotypes 1 and 14 have also been described as highly virulent. Indeed, it has been shown that highly virulent strains of serotypes 1, 2 and 14 in Western countries are mostly included in CC1 (mostly ST1). These strains possess some virulence markers such as the suilysin (SLY), the muramidase-released protein (MRP) and the extracellular protein factor (EF), and presence of genes coding for such proteins can be detected by PCR and this is used in routine diagnosis in some laboratories. Besides this serotype, recent years have seen the emergence of serotype 9 strains among swine diseases in several European countries. Most of these strains isolated from diseased pigs (and differently from those recovered from tonsils of healthy pigs) belong to specific STs (such as ST16, ST123 and ST125) and produce SLY and a variant of the protein MRP. Interestingly, it is not easy to reproduce disease with such “virulent” serotype 9

strains and usually highly aggressive intravenous or intratracheal infections are needed. Intranasal infection does not usually induce disease, even with high susceptible caesarian derived colostrum deprived piglets.

What happens with strains isolated in America? One striking observation is that the percentage of *S. suis* serotype 2 strains recovered from diseased pigs is lower in North America than in other parts of the world. In addition, human *S. suis* disease cases are rarely reported in Canada and USA. In fact, it has been shown that serotype 2 strains in North America are less virulent and genetically unrelated to those causing disease in other parts of the world, such as Europe and Asia. Serotype 9 strains are also different and belong to a bunch of different STs, not usually associated with disease. Highly virulent serotypes 1 and 14 (CC1, ST1) are, however, present in these two countries. On the other hand, most isolates from diseased pigs belong to different serotypes (such as serotype 1/2, the most prevalent in USA). Analysis of virulence markers (MRP, EF and SLY) does not give any additional information since most serotype 2 strains are negative for SLY and EF and serotype 9 strains are negative for SLY. In Latin America, there is not much information and the presence of highly or lower virulent strains of serotype 2 may depend on the source (geographical region) of genetics. Data from Argentina (a country with more of 20 human cases described, the highest number of cases in whole America) indicate that highly virulent serotype 2 CC1 (ST1) strains are widely present, similar to what is observed in Europe. Data from Brazil, the most important swine producer in Latin America, are missing. In addition, no human cases have been reported, which may indicate a problem of misidentification in laboratories of human medicine.

Do *S. suis*-related diseases depend on the presence of virulent strains only?

S. suis-associated diseases are complex. The presence of a potential virulent strain alone does not guarantee appearance of clinical signs and, sometimes, clinical signs are observed in the absence of such strains. Virulence of strains belonging to serotypes other than serotypes 1, 2, 9 and 14 is almost unknown, there are no virulence markers and there is no validated model of infection in pigs, since in most cases, animals infected with strains belonging to other serotypes will not develop disease. However, these strains are commonly found in *S. suis*-associated diseases, North America, respectively. This is still one of the major challenges we face in the diagnosis of *S. suis* infections: how to determine if a given strain (from non-serotypes 1, 2, 9 or 14) isolated from a diseased animal is, in fact, really responsible for most clinical cases in a farm. We usually recommend performing a necropsy of at least 3 animals from the same batch, in 3 different batches. If *S. suis* is isolated in pure or predominant culture from internal organs (other than lungs) in most of these animals, and serotyping indicates that one or a very few serotypes are always involved, we can conclude that those strains are probably important. If inconclusive results are obtained, additional animals should be analyzed: if at the end, 4-5 or more serotypes are detected, predisposaingfactors should be taken into consideration before planning the use of an autogenous vaccine (see below). The analysis of the results is even more complicated if untypable strains are involved: are these all the same strain? Or different untypable strains are causing disease? These questions can only be answered through the sequencing and comparison of such strains.

In fact, when multiple strains are involved, other factors may influence the presence of disease (Figure 2). However, *S. suis* is also the disease of the exceptions, since everything may happen. In rare conditions, 3-4 different virulent strains affecting animals from the same herd (example: serotypes 1, 2 and 14, all ST1, or a serotype 2 ST1 and a serotype 9 ST16) may also occur and this complicate even more the diagnosis. We have recently observed animals dying at the same time with pure culture isolation of a serotype 2 (one animal) and a serotype 14 (the second animal), both virulent strains.

Obviously, the presence of virulent strains is an important factor. However, concomitant infections with other pathogens may highly contribute to the presence of clinical signs. The most important, by far, is the instability of the farm to Porcine Reproductive and Respiratory Syndrome (PRRS) virus. Although data from the field clearly show that a PRRSv previous infection predisposes to clinical disease caused by *S. suis*, the exact mechanisms involved are still unknown. It has been also shown that previous infection with Aujeszky's disease virus may predispose animals to an enhanced *S. suis* disease. Swine influenza virus (SIV) may also enhances the infections caused by certain serotypes of *S. suis* which possess sialic acid in their capsule: serotype 2 but also serotypes 1, 1/2 and 14. How this infection may influence disease caused by other serotypes (which do not have any sialic acid in their bacterial surface) is unknown. Unfortunately, there are no scientific data concerning other co-infections, such as those caused by porcine circovirus, mycoplasma or others. Co-infections (with clinical disease) with *Haemophilus parasuis* seem to be rare. The presence of mycotoxins has also been suggested as a predisposing factor but it has never been scientifically studied.

It is also considered that environmental factors may greatly influence the appearance of *S. suis*-related diseases, such as (among others) poor ventilation, high humidity, inadequate sanitation and important temperature variation between night and day. It is interesting to comment here how experimental infections by the intranasal route are done with virulent strains. If a high concentration of a virulent strain of *S. suis* serotype 2 is inoculated to the nasal cavities of conventional pigs, usually few or no clinical signs are observed. A previous treatment done with acetic acid to irritate the nasal mucosa should be done, followed by the infection with the virulent strain. Under these conditions, clinical

signs may be observed, and this will depend on the farm from where the animals originated. This indicates that other environmental factors, such as high levels of dust and ammonia (causing irritation), may greatly influence animal susceptibility to develop clinical signs. Chances to reproduce disease will considerably increase if some kind of stress is applied to the infected animals. Although *S. suis* may be clearly a primary etiological agent of disease, **the Koch's postulates** sometimes **are not easy to be reproduced** with this difficult pathogen.

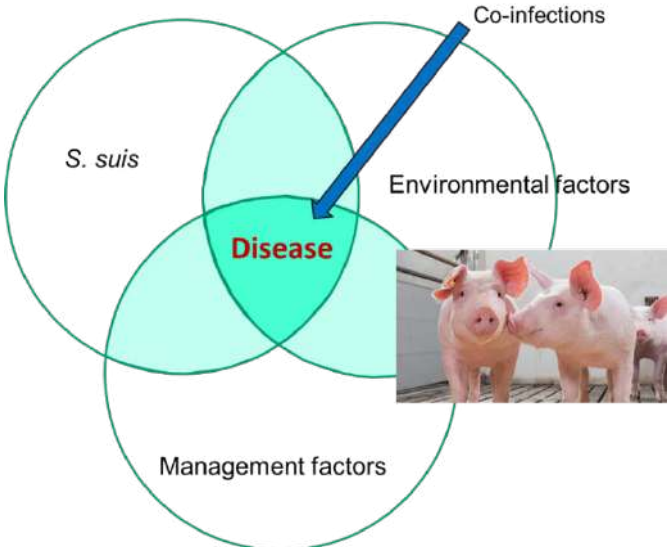

Figure 2: Factors contributing to the expression of *S. suis*-related diseases

Management factors may also influence the development of *S. suis*-related diseases. For example, high level of cross fostering, overcrowding, teeth clipping and tail docking (arthritis), ear notching (arthritis), mixing pigs of different ages, poor adaptation to solid feed in the nursery and low levels of vitamin E. It has been also suggested that the use of strong antibiotics given at the first week of life may been associated with the increased presence of clinical signs due to *S. suis*.

Finally, our studies have demonstrated that the immunological status of animals is extremely important. Indeed, most clinical cases occur when maternal antibodies decline (between 5 and 9 weeks of age) and before natural antibodies are produced. Indeed, natural antibodies slowly increase from 9-10 weeks of age: nobody knows if such antibodies are directed specifically against *S. suis* or to other antigenically-related bacteria present in tonsils. These antibodies are always present in pigs (even in the absence of *S. suis*-related clinical disease in post-weaned animals), increase with age and are probably protective. This may explain why clinical cases are rarely observed in adult animals.

Does *S. suis* represent a danger for antibiotic resistance?

As mentioned before, the incidence of the disease is usually kept under 5% in the field, but this is mainly due to the extensive and routine (where allowed) prophylactic and metaphylactic use of antibiotics. In some countries, where antibiotics in feed are banished, these are sometimes used in water. The problem of the antibiotic use for *S. suis* infections is not necessarily the development of resistance of such strains to the most frequently molecules used to treat *S. suis*-affected pigs. The antibiotics of choice for this type of infection are beta-lactams. Most strains able to cause disease are susceptible to such drugs and this is due to the mechanism of resistance: it is not mediated by enzymatic degradation of the beta-lactam molecules (beta-lactamases), but rather involves modifications in the form of altered molecular weight and/or a decrease in the penicillin-binding capacity of its beta-lactam target proteins: the penicillin-binding proteins (PBP). Indeed, an increase in resistance to penicillin and amoxicillin by *S. suis* is brought by distinct cumulative alterations in its PBPs which happen at the chromosomal level. The consequence of such mechanism of resistance is that it may take years to develop some kind of resistance and, if it develops, it progresses very slowly. On the other hand, it is important to keep testing field strains to confirm such an hypothesis.

It is important to note that some diagnostic laboratories do not use standardized methods to measure resistance, so equivocal results are sometimes observed, as for example: sensitivity to penicillin but resistance to amoxicillin or resistance to amoxicillin but susceptibility to amoxicillin with clavulanic acid: this is simply unlikely to be true. If some kind of resistance to beta-lactams is observed, it is recommended to send the isolate to an independent laboratory to

repeat the test. Some strains isolated from tonsils may present lower level of susceptibility, event to beta-lactam antibiotics: most of these strains are non-encapsulated and non-virulent. In addition, many of these bacteria do not even belong to the *Streptococcus suis* species.

So, can we say that antibiotic resistance is not important for *S. suis*? It should be remembered that some of the antibiotics used are also of importance for human medicine. In addition, worldwide data from resistance of *S. suis* to antibiotics are alarming. Field strains are highly resistant to many different antimicrobials (such as tetracyclines and erythromycin), even if they are still susceptible to beta-lactams. Indeed, *S. suis* is considered **a niche for antibiotic resistance** and represents **a high risk of transmission of resistance** to other pathogens. This arises from **mobile genetic elements** in *S. suis* carrying resistance genes that are transferable at high frequency not only between *S. suis* strains but also **to other bacterial species**. Again, it is important to emphasize the need for continuous surveillance of resistance patterns in all pig pathogenic bacteria.

How to prevent disease caused by *S. suis* without using antibiotics?

Restrictions in the use of antibiotics brought, among other consequences, an increase of clinical disease in post-weaned piglets. In farms where animals are raised without antibiotics, *S. suis* is one of the most important concern. The question everybody is asking: how can we prevent *S. suis* diseases? Everybody also agrees that controlling stress and predisposing factors (concomitant infections, environmental and management factors, etc.) may significantly help to reduce disease. However, this is frequently not enough. What else can be done?

There are many alternatives to antibiotics that have been tested for *S. suis*. However, most of them have been tested *in vitro* but not *in vivo* (Table 1). So far, no active compound has been clearly demonstrated as being effective to control *S. suis* disease *in vivo*.

Table 1: Different products tested as an alternative to antibiotics to kill *S. suis*

Product	**Tested**	**Effect observed**	**Publication (peer reviewed)**
Probiotics	*In vitro*	Yes	Yes
Phages	*In vivo (mice)*	Yes	Yes
Defensins	*In vitro*	Yes	Yes
Bacteriocins	*In vitro*	Yes	Yes
Galabiose	*In vivo*	No	Yes

The use of feed additives became also popular, based on the hypothesis of *S. suis* causing disease through the intestinal route, as discussed above. However, most available studies either have been tested *in vitro* or have not been published in peer-reviewed journals and lack the strict evaluation from the scientific community. Some examples are present in Table 2. Again, the use of these products become more and more popular, but there is no scientific proved data indicating any advantage to use them to control and prevent *S. suis* infections. More controlled and scientific research is indeed necessary.

Table 2: Feed additives tested to control *S. suis* infections

Product	**Tested**	**Publication (peer reviewed)**
Lauric acid	*In vitro*	Yes
Lauric acid	*In vivo*	No
Fatty acids	*In vitro*	No
Cinnamon, oregano, thyme	*In vitro*	Yes
Basil, rosemary, peppermint	*In vitro*	Yes
Cello-oligosaccharides	*In vivo*	No
Alfalfa	*In vivo*	No

As the complexity of *S. suis* epidemiology in swine increases (multiple strains, multiple serotypes), field reports describing difficulty in disease control and management are common. A logical alternative is the use of vaccines. However, so far, there is no commercial vaccine able to protect against all serotypes/strains of *S. suis*. Many research studies evaluated sub-unit vaccines (proteins) or even live vaccines, but controversial results have been obtained. The consequence is that the only alternative practitioners have in hands is the use of bacterins (killed whole bacteria), mostly autogenous vaccines. Autogenous vaccines are bacterins based on the predominant strain(s) recovered from diseased pigs in the affected farm and produced by accredited laboratories. Most published studies have been done with bacterins produced in research laboratories with reference strains, a kind of artificial “autogenous vaccine”, not produced by accredited laboratories.

For the production of an autogenous vaccine, the first step is the choice of the strain. Differently from *Glaesserella (Haemophilus) parasuis*, *S. suis* is easy to isolate. However, under certain circumstances, different strains may be isolated from diseased pigs within the same farm (see above4). *S. suis* may be either a secondary or primary pathogen: as mentioned, co-infections, environmental or management issues may help moderately virulent *S. suis* strains, normally located in tonsils, to induce disease. In these cases, it is better to concentrate the efforts to reduce predisposing factors, as *S. suis* disease is a consequence not really a cause of the health problem. Diagnosis of *S. suis* infection as primary pathogen is not easy. Figure 3 shows a standard procedure that may help on the decision to incorporate a given strain to an autogenous vaccine.

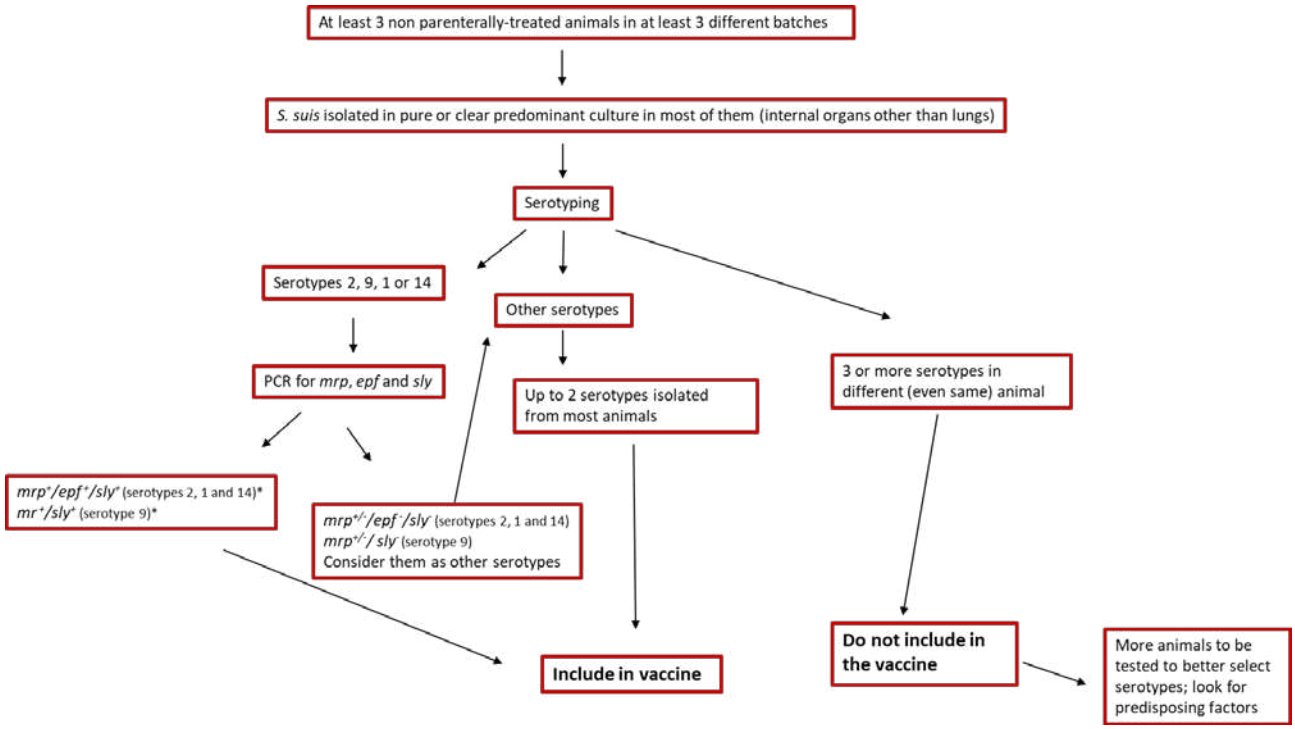

Figure 3: Proposed methodology to choose strains to be included in an autogenous vaccine. *For European strains only.

In some farms, autogenous vaccines include 4-5 serotypes of *S. suis* and sometimes other bacterial species, such as *Staphylococcus hyicus*, *Streptococcus dysgalactiae*, *Glaesserella (Haemophilus) parasuis*, *Erysipelothrix rhusiopathiae* and *Actinobacillus suis*. Although never studied, the inclusion of such huge mass of antigens may have two different consequences: a) the reduction of the bacterial concentration of each individual strain to keep a 2 ml vaccine, and/or b) distraction of the immune system. It is hard to evaluate if all strains are necessary and if the immune system is able to produce antibodies to all relevant antigens. Finally, the production of autogenous vaccines is more an "art" than a "science". The value of autogenous vaccines, at large, cannot be evaluated. Why? Because each company produces the vaccine differently, and most of the variables have never been studied. Some of the variations that may happen among different vaccine productions are presented in Table 3.

Table 3: Some variables that may be present when producing an autogenous vaccine.

Characteristic	**Variables**
Bacterial growth (a)	Exponential vs Stationary
Bacterial growth (b)	Solid medium vs Liquid medium
Bacterial growth (c)	If liquid medium: shaking flasks? biofermentor?
Bacterial growth (d)	Type of medium
Bacterial growth (e)	Aerobic, microaerophilic or anaerobic conditions
Body of the vaccine	Washed bacteria or bacteria + supernatant
Bacterial concentration	High vs Very high; keep concentration when several serotypes included?
Inactivation procedure	Formalin vs others
Adjuvants (a)	Type of adjuvant: type of immune response?
Adjuvants (b)	Concentration?

Indeed, under each condition of vaccine production, different antigens may be expressed. In addition, it has also been demonstrated that adjuvants used may greatly influence the protection observed with experimental vaccines. So, it is impossible to compare autogenous vaccines produced by different companies. This explains in part why there are almost no scientific data evaluating autogenous vaccines in general in the field, at least published by peer-reviewed journals. Most data are from internet or oral presentations in different congresses and other meetings. In addition, most studies do not include control groups: the contribution of the autogenous vaccine to the control of the infection is normally evaluated by studies done "before" vs "after", where mortality and the use of antibiotics to treat animals are compared. One of the problems is that, normally, "mortality" refers to total mortality in the nursery…not necessarily mortality related to *S. suis* only. In addition, other measures to control predisposing factors may also be applied simultaneously with vaccination, which may complicate the analysis. On the other hand, the inclusion of a control group may not solve the problem: when mortality is under 5%, a significant high number of animals must be included in both groups, since otherwise it is difficult to observe differences. Finally, there are no studies where the antibody response of vaccinated animals with a commercial autogenous vaccine have been evaluated, so it is unknown if such vaccines are able, at least, to induce an increase of the antibody levels, which would be an indirect way to study potential protection.

As mentioned, adjuvants can dramatically influence the vaccine-induced antibody response, as it was studied with experimental (not commercial) vaccines. Not all antibodies (immunoglobulins or IgG) induced by a bacterin are indeed protective. Some IgG subclasses (called "isotypes"), such IgG2 and to a lesser extent IgG3, are particularly effective at mediating bacterial ingestion and destruction by leukocytes (phagocytosis). Indeed, *S. suis* resistance to phagocytosis and thus innate immunity clearance, is lost in the presence of antibodies that promote bacterial phagocytosis and destruction by professional phagocytes (called "opsonophagocytosis" or OPA test). Other antibodies isotypes, such as IgG1, even if they are induced after vaccination and recognize the pathogen, are much less protective, since they cannot help the host to destroy the pathogen. Interestingly, different adjuvants may influence the production of "protective" or "non-protective" IgGs. An *in vitro* OPA test has been lately standardized to measure protective activity of antibodies against *S. suis*, although it has never been used so far to measure the antibody response against field autogenous vaccines. So, not only levels of antibodies should be measured, but also their functionality. We are presently developing such tests in our laboratory and results will be available soon.

Finally, there are no clear data when and how autogenous vaccines should be applied, and there are almost no scientific studies available. In the field, and without any scientific data, autogenous vaccines are used either in sows or piglets or (less common) both. Vaccination of sows before farrowing might elicit passive maternal immunity, being less costly, and thus representing an economical alternative to piglet vaccination. Yet, available results indicate that pre-farrowing immunization of sows with these experimental bacterins to protect piglets gave inconclusive results with either no antibody production, antibody production that did not protect piglets or a restricted protection for piglets of less than 6 weeks of age. In fact, piglets at nursery remain without antibodies, a period of high risk of *S. suis* disease in many farms. Active vaccination of young animals, such as suckling piglets, has the concern of possible interference with maternal antibodies. Indeed, neither vaccination of suckling nor of weaning piglets from immunized sows with experimental bacterins was constantly associated with a prominent active immune response and protection at 8 weeks of age. In this regard, interference between maternal antibodies and active production of antibodies against *S. suis* was also suggested in a field study with also an experimental bacterin. Vaccination of older piglets (for example, at 3 and 5 weeks of age) may not induce a booster antibody production early enough to protect piglets at the nursery: there is clearly a problem of a window of vaccination. Knowledge of antibody kinetics is thus required before implementation of a rational vaccination program. The adopted strategy should allow minimal interference between passive maternal immunity and active immunization in piglets but maximal protection for pigs at the approximate time of onset of clinical signs.

Conclusions

S. suis infections are multifactorial and very difficult to control. With the reduction in the use of antibiotics, nursery mortality due to this pathogen significantly increased. *S. suis* may be a primary or secondary pathogen and control of predisposing factors should not be neglected. Evaluation of the virulence of the involved strains is not easy to perform under all circumstances; in addition, the virulence potential of the strain is only one of the aspects to be taken into consideration to control disease. Products that can be used as alternatives to antibiotics still need to be scientifically evaluated. Finally, due to limited field reports concerning immunogenicity and protection, the usefulness of autogenous bacterins in a rational vaccination program remains to be proved and needs further research. Studies where independent researchers evaluate the influence of different aspects of the production of an autogenous vaccine on their protective capacity are also required. As it is still the only option available to be used as immunogens, a diagnostic effort on the evaluation of the strains to be included in such vaccine must be done. Finally, autogenous vaccines must be produced by companies having a large experience on this field.

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What do we still have to learn about *Mycoplasma hyopneumoniae* infections in pigs?

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Introduction

Talk about traditional bacteria in pigs, and *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) will be among the first ones to be mentioned. The "tradition" of this microorganism may be based on the fact that it has been recognized as a swine-specific pathogen for more than five decades, that it is prevalent in most areas where pigs are raised, and that it is common to accept its circulation in pig herds. Nevertheless, our understanding of this bacterium and the disease it causes has changed significantly in recent years, and keeps changing as you read this document. Thus, it is clear that we still have lots to learn regarding *M. hyopneumoniae* infection in pigs.

Some aspects remain unchanged and are still challenging

Regardless of its significant importance in swine production, progress on the understanding of *M. hyopneumoniae* has been hindered due to several factors:

- *Mycoplasma hyopneumoniae* is a bacterium only affecting swine. The bacterium has been detected via PCR in the nose of humans exposed to pigs. However, there are no reports in the literature suggesting that this pathogen affects any other host species. Thus, the methods and research tools commonly employed for *in vitro* investigation on this bacterium have not benefited from assay development in other host species, as it can be the case with multi-host pathogens. For example, the lack of commercially available monoclonal antibodies is a significant limiting factor for *M. hyopneumoniae* research advancement.
- Being a bacterium, one could assume that classic bacteriology would be sufficient to detect the microorganism in tissues, pig fluids and secretions, or in the environment, in order to obtain field isolates and to characterize them. Nevertheless, *M. hyopneumoniae* has proven to be a challenging pathogen to work with in laboratory, as well as in field conditions. Always considered a hard to be grown pathogen, *M. hyopneumoniae* bacterial isolation is seldom requested due to a very low success rate. It is important to mention that recent comparative studies have investigated the potential to develop methods for improved bacterial isolation, like specific media for bacterial growth. However, new recipes for improved Mycoplasma media are not currently publicly available.
- As a pathogen, *M. hyopneumoniae* can cause disease by itself. However, poly-microbial infections are very common when observing the clinical signs associated with *M. hyopneumoniae* infections. Concomitant infections with other respiratory bacteria and/or viruses, in the form of Porcine Respiratory Disease Complex is extremely common. This particular feature has made the assessment of the clinical effect of *M. hyopneumoniae* infections complicated, hard to pinpoint and difficult to quantify. The lack of accurately determined disease costs for swine production has allowed for an underestimation of the health problem this bacterium causes and leads to the use of partially efficient control measures.
- Infections with *M. hyopneumoniae* are highly prevalent and ubiquitous. Although this is a reality that can change significantly in the future, infections with this bacterium are considered present in a majority of farms, regardless of the type of production system and the geographical location. This aspect remains a significant drawback for disease control.

Changes have occurred in recent years

It is common to hear that *M. hyopneumoniae* has changed, that the disease problems associated with its infection can be more severe and less "controllable" in the field. While this hypothesis could be true, one can argue that the way we raise pigs has changed as well, and that the pig itself is different, but one other aspect that is significantly important is the fact that we have changed what is understood of *M. hyopneumoniae* infections.

Mainly, a shift in the state of knowledge of *M. hyopneumoniae* epidemiology has been evident in the most recent decade. A disease formerly considered exclusive of the growing pig has become focus of control strategies applied at the sow farm. Identification of risk factors in the sow farm has led to the development of targeted tools, allowing producers and veterinarians to generate stability in sow farms and harvest the benefits of raising piglets that are minimally colonized (and sometimes even negative) to the bacterium.

It is important to recognize that progressive swine medicine and production has been key to the adoption of new knowledge and application of innovative tools for disease control. However, there are many unanswered questions regarding *M. hyopneumoniae* infection in pigs, which should be addressed in order to move disease elimination efforts forward.

What we still have to learn

Doubtlessly, a comprehensive list of unresolved aspects regarding *M. hyopneumoniae* and the infections it cause would be long. Here, several factors, which may have key implications for the course of disease presentation, are listed:

- A significant amount of information has been generated in recent decades showing that the virulence of *M. hyopneumoniae* strains differ. The difference in virulence is expressed in various ways, from laboratory assays, to clinical presentation, to lack of efficacy of control measures. Mainly, differences in virulence have been assessed in experimental settings, which allows for proper characterization of strains. However, a practical assay, which could be applied in the field, indicating the capability of *M. hyopneumoniae* to cause more severe disease is not available to-date. Development of such diagnostic capacity would be crucial to define specific measures directed at disease control. In ideal situations, one could characterize the *M. hyopneumoniae* strain circulating on a herd, be capable to accurately predict disease presentation and apply specific treatments depending on strain virulence.
- Similar to bacterial virulence, the genomic difference in *M. hyopneumoniae* strains is frequently observed in the field and in the laboratory. Several methods, such as sequencing and typing, have been standardized for the characterization of clinical specimens and have allowed for the analysis of samples from distinct geographical locations, production systems and times. To date, molecular characterization data has been used mainly to aid comparing strains for outbreak investigations. However, inferring evolution or predicting of cross-protection has not been accomplished employing current methods evaluating strain differences.
- An extremely long persistence in the host (surpassing seven months) is now widely recognized as part of the epidemiology of *M. hyopneumoniae*. Importantly, the infectious capability of persistently infected pigs has been documented and constitutes a silent risk factor for pathogen circulation in pig farms. Remarkably, pigs may not even show clinical disease during this chronic infection phase. Efforts have been made to attempt shortening the persistence period. The use of vaccine and antimicrobial agents has been explored on this scenario, but found to be unsuccessful. However, factors driving *M. hyopneumoniae* persistence in the host remain unknown and are a major limitation to the design and application of preventive measures or treatments to decrease the infectious period.
  A long infection period has different implications based on the production system and phase pigs are raised in. For example, growing pigs are likely to be positive to (and shed) *M. hyopneumoniae* at the end of the growing period if they were infected earlier in life. On the other hand, the implications of *M. hyopneumoniae* infection timing are far more crucial for gilts and sows, which are housed for years in pig farms. A late *M. hyopneumoniae* infection, combined with a long infection period, can lead to shedding of the bacterium during farrowing and lactation periods, which can translate into exposure of the offspring to *M. hyopneumoniae*. Thus, gilt acclimation practices specific for this bacterium are gaining popularity, especially in North America, in order to generate stability in breeding herds.
- Gilt acclimation for *M. hyopneumoniae* can be challenging based on several of the limiting factors mentioned above. Disease preventive measures are usually partially protective and do not prevent colonization with this bacterium. Antibacterial treatments help easing problems associated with clinical presentation, but do not lead to complete elimination of *M. hyopneumoniae* from the host. Thus, purposeful exposure of gilts to *M. hyopneumoniae* at a young age, allowing ample time for recovery from disease, is becoming a standard practice in some countries. It is important to note that gilt purposeful exposure is a new method that needs to be further validated for safety and efficacy. Therefore, the development and validation of science based protocols for this purpose has to be a priority for the industry.
- A standing question involving immunology, epidemiology, and disease elimination is whether previously infected pigs are protected from reinfection for life, or whether they can be reinfected with *M. hyopneumoniae* once the persistency period has ended. Resistance to reinfection is assumed for this disease, although little data can be identified in the literature addressing this question. One of the assumptions of current *M. hyopneumoniae* eradication protocols is resistance to reinfection. For that reason addressing the reinfection issue results critical for total *M. hyopneumoniae* disease control, although it may not be simple to accomplish.
- The fact that diagnostics for *M. hyopneumoniae* are less than ideal cannot be ignored. Practitioners and investigators have summed efforts and have evaluated novel (and non-conventional) samples and have combine it with highly accurate methods for pathogen detection. Still, *M. hyopneumoniae* is stealth and hard to detect in many situations. Simple sample collection with a high degree of sensitivity and specificity for *M. hyopneumoniae* detection is certainly in the wish list for swine health professionals. In addition to detection, take the development

of assays for evaluation of antimicrobial susceptibility without the need to perform bacterial isolation or the ideal situation to differentiate exposure from infection using ELISA based tests.

- Certainly puzzling, the isolation or detection of *M. hyopneumoniae* in tissues and body sites other than the respiratory tract are becoming more frequent. Whether this a mere reflection of improved diagnostic capabilities, the potential implications of such findings can be substantial in swine production. For example, addressing biosecurity can become extremely challenging when a pathogen can be hidden in unexpected locations in the body of the host.

Closing remarks

Regardless of the fact that it is still considered limited, the knowledge on *M. hyopneumoniae* as a pathogen and its associated disease has grown significantly in recent years and has allowed swine professionals to propose novel approaches for disease control and eradication. However, more information is certainly needed to reach a common health objectives regarding *M. hyopneumoniae* control and eradication.

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Science of biosecurity: understanding the mechanisms of pathogen entry into pig populations, and how to implement effective biosecurity improvement programs

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Introduction

The global swine production industry has experienced a revolution in the last 30 to 40 years. Forty years ago, production was geographically centralized (i.e., all production activities were centrally located), small scale and outdoors. Producers were knowledgeable in many areas, but a specialist in none and few had employees. Swine production has now transitioned to where many of the production activities are geographically decentralized at specialized facilities. The stages of production are geographically segregated where the breed-to-wean, nursery (or rearing) and finishing (or fattening) stages of production occur at separate locations. Other examples include boar studs where semen is collected, processed and delivered to sow farms and large-scale feed mills that mill and deliver feed to many farms. Small scale, outdoor production has been replaced by large-scale systems where pigs are raised entirely indoors. There are now over 31 production companies globally that have at least 100,000 sows (Hess, 2019). The size of individual farms has also grown. In the United States, for example, 27 percent of swine farms were considered large (>5,000 head) in 1994 (USDA, 2015). By 2014, just 20 years later, over 93 percent were considered large. As the size of farms and production companies increased, they became more specialized and more reliant on employed labor. On many swine farms, the owner(s) no longer regularly works on the farm. The larger production companies now hire very specialized labor, including veterinarians, nutritionists, reproductive physiologists, and animal welfare specialists. The larger production companies have opportunities to share equipment, such as livestock trailers and feed trucks, to use these resources more efficiently (i.e., not sitting idle) and capture significant economies of scale. What have these changes meant for biosecurity? All have resulted in major increases in the size of losses when pathogens are introduced into a herd and the frequency of opportunities to introduce those pathogens. It is not uncommon in herds with at least 4,000 sows to have losses that exceed US$1,000,000 after an outbreak of PRRS. The various forms of specialization and geographic segregation of production and ancillary production activities (e.g., feed mills, boar studs and gilt multiplication farms) have led to a major increase in the frequency of events where pathogens can be introduced into a herd. A list of examples, that is by no means all-inclusive, includes feed, semen and replacement gilts that are routinely delivered to sow farms, and with them the drivers, vehicles and trailers that deliver them.

Swine diseases and disease management have also evolved in the last 30 to 40 years. The emergence of PRRSV in the 1980s was arguably the most significant swine disease event to occur in most of our lifetimes. The recent spread of African swine fever virus (ASFV) may alter our collective opinions about that in the next decade. The adoption of all-in-all-out production of growing pigs and the emphasis on eliminating swine pathogens, such as porcine reproductive and respiratory syndrome virus (PRRSV), porcine epidemic diarrhea virus (PEDV) and *Mycoplasma hyopneumoniae* (*Mhp*) from sow herds have also increased the value of biosecurity. When groups of growing pigs are negative for certain pathogens and lacking immunity at placement, the value of keeping those pathogens out is immense. When investments are made to eliminate pathogens from sow farms, the value of preventing the reintroduction of the pathogens is enormous. In the case of PRRSV, where heterologous immunity is often sub-par, the value of not introducing new isolates into sow herds is large, even if the herd is already infected with other isolates.

For countries that are presently free of specific pathogens, such as ASFV, foot and mouth disease virus (FMDV) and pseudorabies virus (PRV) in the United States, the need to prevent these transboundary pathogens from entering and spreading rapidly has also increased in importance as the world has become more connected and global trade more important. The recent spread of ASFV in Southeast Asia and Eastern Europe has put the world pork industry on high-alert.

While the need for biosecurity on swine farms has increased, the industry has been slow to respond to the new calculus. Why has progress been slow? For the last 15 years, I have focused much of my research and outreach on biosecurity, biosecurity risk assessments and outbreak investigations. During that time, I have observed swine producers and veterinarians use a "ready-fire-aim" approach to biosecurity. I have been guilty of it myself. However, is "ready-fire-aim" the right order? The answer is not readily apparent. Entrepreneurs are frequently celebrated and rewarded for approaching things in this order, especially with disruptive technologies where being first-to-market is everything. However, is it the right order to approach biosecurity? I believe that it has led to misallocation of resources and very slow progress on improving biosecurity and reducing the frequency of outbreaks of PRRS and other diseases.

The ready-fire-aim approach has been guided by experimental research. When a new study was published demonstrating that, for example, livestock trailers, boots, insects or aerosols are capable of carrying pathogens from herd-to-herd, the industry has opened fire with only a rudimentary understanding of how much livestock trailers, or boots, or insects or aerosol were contributing to the herd-to-herd transmission of pathogens. Just because they can, does not mean that they frequently do. Were resources being spent on these when they would have been better spent addressing other things that can carry pathogens from herd-to-herd?

The better order is "ready-aim-fire." What does the aim step in ready-aim-fire look like? Very simply, it is trying to identify the most frequent ways by which pathogens are being introduced into herds and then prioritizing improvements in biosecurity to address them. It is taking time to identify the most significant vulnerabilities on farms to determine what should be done next. Experimental research studies are no help for identifying the most frequent ways that pathogens are introduced. To study this question, we have to observe what is happening. However, ad-hoc approaches to biosecurity risk assessments and outbreak investigations are not sufficient. Learning faster from observing requires a more systematic, comprehensive and consistent approach.

My experience with the Production Animal Disease Risk Assessment Program (PADRAP) (Holtkamp et. al., 2012) in the early 2000s left me frustrated. The survey was useful for identifying specific risk factors that were present or absent and specific biosecurity practices that were done or not done. That may have provided some sense of what to fire at; however it offered little in the way of prioritizing (i.e., aiming). In PADRAP, a score was assigned to each response to the questions which conveyed a sense that some risk factors or practices were more important than others. The aggregated risk scores in PADRAP did prove to have some value for predicting herds where introduction of PRRSV, causing outbreaks, occurred more frequently. But the risk scoring system had several shortcomings. First, the score assigned to a specific response was independent of the response to other questions in the survey. For example, the score for not washing livestock trailers between every load of weaned pigs was the same whether the trailer was dedicated to the farm or used to haul pigs from 20 other sow farms. The score for any given response was also independent of a host of other information that was not addressed in the survey. For example, if one of the trailers that hauled weaned pigs was driven by someone who also worked at a market where culls sows were commingled. There is no survey that does, or ever will, account for all of the important information for every farm under every circumstance. That is also why large observational studies where surveys are used to collect data on biosecurity practices and risk factors give conflicting answers about the most significant practices and risk factors on farms (Holtkamp et.al., 2010; Holtkamp et. al., 2012; Bottoms et. al., 2012). PADRAP was also poorly organized according to a haphazard combination of pathogen carrying agents, such as trucks and people, and events, such as entry of semen and entry of employees, which occur on swine farms. PADRAP was not very helpful for identifying the most frequent ways by which pathogens were being introduced into herds. It did not help prioritize where to spend time and other resources next.

Methods: A different approach

Experience with PADRAP also taught me that producers and veterinarians were most interested in assessing biosecurity and taking action to improve it in the aftermath of an outbreak. It is human nature to be most responsive in the face of a crisis or threat. It makes a great talking point to say that we should not wait for an outbreak to improve biosecurity, but it rarely works that way. With funding from the Iowa Pork Producers Association, my students and I developed an approach to conduct epidemiological investigations of outbreaks in a systematic, comprehensive and consistent manner. The resulting approach, and forms used to guide the approach, has also proven valuable for conducting risk assessments prospectively.

The approach developed was based on the basics of epidemiology and risk assessment and considered how swine pathogens are transmitted from one herd to another. Since bacterial and viral pathogens are not capable of locomotion, something must carry the pathogens from one herd to another. The term “pathogen carrying agent” was applied to any object, animal, or person that may carry a virus into the breeding herd. A pathogen carrying agent may directly transmit the pathogen by being infected or indirectly by being contaminated with the pathogen. For epidemiological investigations of outbreaks and prospective risk assessments, the objective is to identify vulnerabilities on the farm being investigated or assessed. Therefore, the investigation or assessment is made from the perspective of the farm being investigated or assessed. Imagine standing in the middle of the farm, observing the potential pathogen carrying agents entering the farm. The entry of pathogen carrying agents, delineated by when they cross the outer perimeter of the farm, can be characterized as events. A "carrying agent entry event” is defined as an event where one or more potential pathogen carrying agents crosses the outer perimeter, physical or abstract, of the swine farm. Carrying agent entry events that typically occur on sow farms include delivery of semen, entry of breeding replacements, removal of cull animals, removal of weaned pigs, disposal of carcasses, delivery of feed, collection of garbage, delivery of supplies, entry of on-farm employees, entry of repair/service personnel working inside barns, entry of repair/service personnel working outside barns, entry of other visitors, entry of food, removal of manure, entry of other animals (non-swine domestic and wild), entry of insects, and entry of air and water. Some carrying agent entry events, such as entry

of air and water, occur continuously. For discrete events that are easily observed and recorded, such as entry of semen and removal of manure, a date(s) or frequency over some period of time can be determined. For others that are discrete but not always observed, such as entry of rodents and other wild animals, a subjective assessment of how frequently or intensively they occur can be made.

Organizing epidemiological investigations and risk assessments by carrying agent entry events has proven to work very well. PADRAP and many of the biosecurity risk assessment surveys available today are confusingly organized by combinations of pathogen carrying agents and carrying agent entry events. It is essential to be consistent. The alternative is to organize them by pathogen carrying agents consistently. However, there are too many potential pathogen carrying agents that enter farms; over one-hundred on sow farms depending on the level of resolution used to define them. Organizing epidemiological investigations and risk assessments by pathogen carrying agents becomes overwhelming.

Concepts of Hazard Analysis Critical Control Points (HACCP) were also incorporated. HACCP is a system that was initially developed in the 1950s by a team of scientists in the United States to ensure food safety for the manned space program. In HACCP, critical control points (CCP) are steps in a process where failures lead to adverse outcomes, such as the introduction of a pathogen into a herd. In the context of outbreak investigations and risk assessments, identifying the steps in a process, such as entry of semen, and the opportunities for failure is necessary to identify the vulnerabilities in biosecurity on the farm being investigated or assessed. A CCP is also defined as a step where a biosecurity practice (i.e., control) can be applied to prevent a failure or reduce the probability of failure. In the swine industry, the logistics of production are highly variable. For example, if asked to describe the steps involved in collecting, processing, delivering, entering semen into a farm and using it to inseminate animals, most veterinarians would have to think about how it is done on a farm single with which they are familiar. If asked to describe the steps for another farm, even if it is in the same company, they would likely be different. Therefore, to conduct effective epidemiological investigations and risk assessments, where the purpose is to identify vulnerabilities for a farm, the steps involved with each carrying agent entry event, and opportunities for failure must be understood and documented for that farm. That is the basic principle on which the approach we developed to conduct epidemiological investigations of outbreaks and risk assessments was built.

One thing that is consistent for every farm is that the same series of failures is required for a pathogen to be introduced into a herd by a pathogen carrying agent. A biosecurity failure at a single step is necessary but not sufficient. Instead, a series of 3 failures is required for a pathogen to be introduced into a herd by a pathogen carrying agent (Fig. 1). First, the pathogen carrying agent must be contaminated by, or infected with, the pathogen before entering the breeding herd. Second, the biosecurity measures in place to mitigate the infection or contamination of that carrying agent must fail. Third, the infected or contaminated carrying agent enters the farm and the pathogen is transferred from the carrying agent to a pig within the herd.

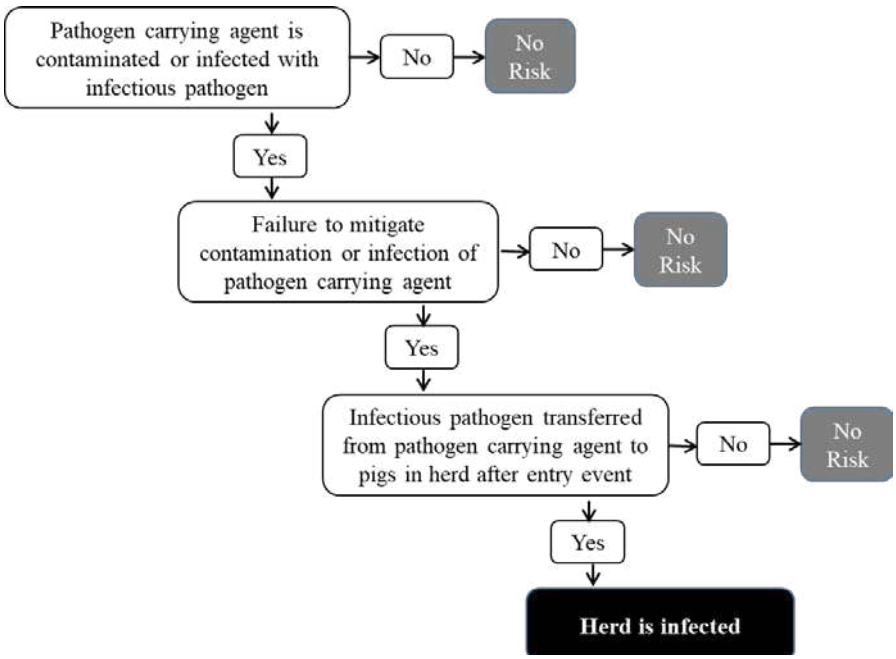

Figure 1. The series of failures required for a carrying agent to carry a pathogen into a herd.

The epidemiological and HACCP principles were used to develop a standardized investigation form. The investigation form for sow farms contains a section for each of the carrying agent entry events that typically occur on sow farms. There is also a section for characteristics of the site, characteristics of the area surrounding the site (up to 7.5 km) and characteristics of the herd.

The information collected for epidemiological investigations of outbreaks and risk assessments includes the frequency (and dates for outbreak investigations) with which each carrying agent entry events occurs. Information on all of the CCPs or steps where the failures may occur resulting in the introduction of a pathogen is gathered through an open-ended discussion. Closed-ended questions are included in the form to gather relatively standard information about the steps involved with each carrying agent entry event and the likelihood of failures occurring at each step. The closed-ended questions also help guide the open-ended discussion. For example, closed-ended questions in the section on entry of semen about testing of boars in the boar stud serve as a reminder to ask about all the relevant details related to how the testing is done and whether the results of the testing are always known and communicated before the semen is used. The closed-ended questions will never capture all of the relevant information, so it is important to get a good narrative of the steps and the likelihood of failures occurring in order to assess the biosecurity vulnerabilities fully. Responses to the closed-ended questions can also be aggregated for analysis of data from multiple investigations and assessments.

Results

As an example of how epidemiological investigations of outbreaks conducted in a systematic, comprehensive and consistent manner, can help to identify carrying agent entry events that are most frequently making farms vulnerable to the introduction of pathogens, nineteen cases of PRRS outbreaks in swine breeding herds were investigated. The herds were located in the Midwest US and all of the investigations were completed between January of 2015 and September of 2017. To assess how the virus may have been introduced into each herd, the investigations were conducted, using the form developed, for a 4-week investigation period ending on the date clinical signs were first recognized. Diagnostic results confirmed that each case occurred as the result of a new isolate of PRRSV in all of the cases, as determined by the herd veterinarian.

For every case, the investigation interview was conducted by a facilitator and an assistant facilitator who took notes and captured all relevant information in the investigation and report form. The same facilitator (Holtkamp) conducted all 19 of the investigations, with three different assistant facilitators. In addition to the facilitators, the herd veterinarian and the farm manager were present at the investigation interview for all of the cases.

The investigation and report form served as a guide for an open-ended discussion of each case and all of the carrying agent entry events that occurred in the four weeks preceding the first clinical signs. The facilitators subjectively assigned each carrying agent entry event a rating low, medium, or high for the likelihood of PRRSV introduction into the herd. The assessment was based on how frequently and when each carrying agent entry event occurred during the investigation period, the steps associated with each event where failures may occur (i.e., CCPs) and how likely the series of failures required for a pathogen carrying agent to introduce a pathogen into a herd occurred (Figure 1). All pathogen carrying agents associated with each carrying agent entry event were assessed.

For the 19 cases investigated, the number of times each risk event was rated medium or high for the likelihood it was responsible for the introduction of PRRSV is summarized in Figure 2. Entry of on-farm employees was the entry event rated high most frequently; rated high 9 times and medium 5, out of the 19 cases. Removal of cull sows and entry of repair personnel working inside the barns were rated high next most frequently.

For the cases where on-farm employee entry was rated high, significant vulnerabilities were identified. On-farm employee entry was the most frequent risk event on all of the farms investigated, creating many opportunities for failures to occur. The average number of discrete carrying agent entry events that occurred during the 28 day investigation period was 369 events per farm, of which 170 (46%) were on-farm employee entry events. A single employee entering the barns was considered one risk event. All of the farms had a shower, but 4 lacked a well-defined clean-dirty line and only 7 had a bench in addition to the shower. Biosecurity practices that were recommended to address the biosecurity vulnerabilities in these cases included the addition of well-defined clean dirty lines and installing benches. The bench adds a second clean-dirty line and serves as an additional layer of biosecurity. It is impossible to fully know or control where employees go while away from the farm, but attempts at some control were observed. In 7 of the 19 cases, a rule that employees were not allowed to visit or work on other swine premises was in place at the time of the outbreak. In 9 of the 19 cases, at least one on-farm employee did visit another swine farm during the investigation period. Only 8 of the farms required downtime for employees after visiting or working on other swine farms and none required employees to wash and disinfect their vehicles. Rules prohibiting employees from living with people that worked on other swine farms were in place at 10 of the farms at the time of the outbreak, but 6 of the farms allowed employees to work in other swine related activities, such as hauling pigs, loading market pigs, delivering gilts, managing a feed mill or delivering feed. Other frequent observations were towels on the dirty side of the shower, shoes worn into the shower area and dirty entryways and showers. High employee turnover, disgruntled employees and managerial transitions were also factors in 4 of the cases. One case, in particular, exemplified how employee issues can

put farms at risk. At the time of the outbreak, the farm manager was transitioning to a new job; several employees had been observed intentionally violating biosecurity rules; two were suspected of having left in the middle of the workday, without permission, to interview on another swine farm and employee turnover exceeded 100% in the year before the outbreak. Because it is difficult to know or control where employees go while away from the farm, attempts to reduce the probability that employees or their vehicles become contaminated (i.e., the first failure) are often less productive. Most producers and veterinarians feel they have more control over practices to mitigate the contamination (i.e., the second failure) by adding more layers of biosecurity, such as benches, hand washing stations, and showers, and focusing training efforts on improving compliance with those practices.

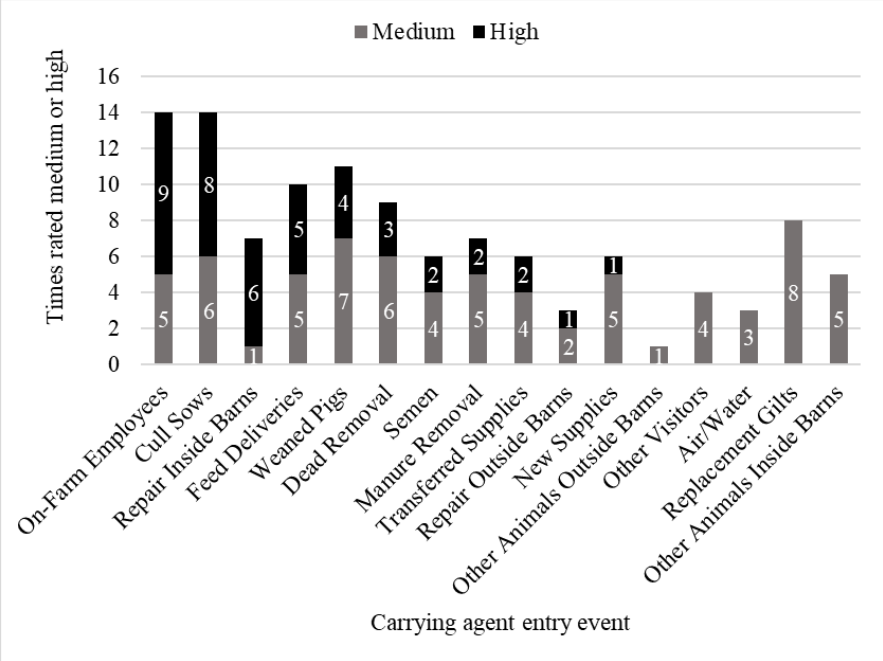

Figure 2. Number of cases, out of 19, that each carrying agent entry event was rated medium or high for the likelihood it was responsible for the introduction of PRRSV.

Conclusions

The dramatic transformation of the pork industry in the last 30 to 40 years has changed the need for, and value of, biosecurity on swine farms, but progress in slowing the transmission of pathogens from herd-to-herd has been slow? In the United States, this was very evident in 2013 and 2014 when PEDV was first introduced into the country. Within 18 months of the initial case, nearly half of the sow farms in the country became infected (University of Minnesota, 2020). The ad-hoc approaches to biosecurity risk assessments and outbreak investigations have not been sufficient. While it is true that mistakes are opportunities to learn, the learning part is not guaranteed. Learning faster from our mistakes requires a more systematic, comprehensive and consistent approach like the one described in this proceedings paper. The approach and form are being used as part of a Rapid Response Program funded by the Swine Health Information Center (SHIC) in the United States. The Rapid Response Program is a nationwide network of veterinarians, state animal health officials or representatives and epidemiologists who are trained, prepared and committed to act within 24 hours of contact to conduct epidemiological investigations when a new transboundary or emerging disease threat occurs with a known etiology. Resources developed for the Rapid Response Program, including the form for conducting an epidemiological investigation of outbreaks, are available at the Swine Health Information Centers website (Swine Health Information Center, 2020).

Acknowledgments

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Strategies to minimize the risk of pathogen spread via feed

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Introduction

As African swine fever virus (ASFV) continues to spread across Southeast Asia, classical swine fever virus (CSFV) expands within Japan, and foot and mouth disease virus (FMDV) reports continue from China, there is increased concern that foreign animal disease (FAD) may enter previously naïve areas. Their continued entry would be devastating to the global swine industry, but also to those that produce feed and ingredients fed to pigs. In their May 2019 Food Outlook Report, the Food and Agriculture Organization of the United Nations reported that pig feed sales were down 10 to 50% in the Guangdong Province of China, and pig feed production in the Shandong Province was only 67% of the volume of 2018 (FAO, 2019).

Risks for Foreign Animal Disease Entry through Ingredients

There are many potential routes for FAD entry into naïve areas, with ingredients being just one. Many entities are taking steps to limit entry through more direct methods, such as regulating the importation of live animals or smuggling of pork products, but it is the responsibility of the feed industry to minimize the potential for FAD entry through a feed vehicle. For FAD entry via an ingredient to occur, there would need to be an initial contamination event, virus survival during transport, and consumption of virus at a dose that is capable of causing infectivity.

Contamination of an Ingredient

Several examples of introduction of FAD via the feed supply chain exist. Introduction of FMDV into Japan and South Korea have both been linked to feedstuffs (Sugiura et al., 2001; Park et al., 2014). The feeding of silage that was harvested from areas with wild boars infected with CSFV has led to illness in naïve pigs (Ribbens et al., 2004). Just five years ago, porcine epidemic diarrhea virus (PEDV) was rapidly spreading throughout the U.S. swine industry for the first time, causing high mortality in young piglets in 27 states. The root cause investigation for PEDV entry in the USA concluded the most likely cause was 1-ton polyethylene tote bags containing feed or ingredients from China (USDA 2015). Feeding contaminated feed has been categorized as a risk factor for ASFV transmission (EFSA, 2014; Belyanin, 2013). This report documented feed being associated with 35% of 284 reported outbreaks in Russia. Contaminated feed has been reported that 35% of the 284 ASFV outbreaks in Russia were linked to contaminated commercial feed. More recently, reports indicate that 1 to 2% of tested ingredients from modern Chinese feed mills were ASFV-positive, including corn, soybean meal, rice, wheat, and corn dried distillers grains with solubles (Dee and Niederwerder, 2019). Senecavirus A (SVA), a swine pathogen that causes similar clinical signs as FMDV, is endemic in swine production systems in the U.S. and was recently found to be spread via SVA-contaminated ingredients and feed in Brazil (Leme et al., 2019). The source of virus entry into the feedstuff is oftentimes FAD-containing feces, which may be introduced into the ingredient through cross-contamination during grain drying or ingredient transportation. Alternatively, in our team's work with a swine production system in Vietnam, none of the 40 feed or ingredient samples collected contained detectable ASFV DNA. Notably, the feed mills in this production system were all using a formaldehyde-based additive. These contrasting cases illustrate that there are clearly differences in the prevalence and distribution of ASFV in ingredients, and many factors must be considered when evaluating ingredient contamination.

Virus Survival during Transport at Concentrations Capable of Causing Infection

Theoretical survival of FMDV (via a Senecavirus A surrogate) and ASFV has been demonstrated by Dee et al. (2018), while CSFV does appear to be as likely to survive transportation. Niederwerder et al. (2019) reported that pigs consuming ASFV-contaminated feed can become infected when consuming a single 100 g meal of feed containing a single dose of ASFV (10^4 $TCID_{50}$/g) ASFV. By modelling this data, it is expected exposure of pigs to 30 meals containing a low dose of ASFV (10^0 $TCID_{50}$/g) will lead to a significant probability of infection. Feed industry equipment and processes are designed to efficiently mix low inclusion products uniformly throughout a batch of feed, and then deliver feed in a method that provides multiple meals with the same consistency to many pigs housed in the same location. If ASFV or another FAD enters the feed supply chain and is mixed into a batch of feed, the results could be catastrophic contamination across multiple herds. For example, Schumacher et al. (2016) reported that if 1 g of feces

from an acutely infected pig with porcine epidemic diarrhea virus (PEDV) entered a receiving pit, it could potentially contaminate 500 metric tons of feed, with each gram having a dose capable of causing infectivity. That is the equivalent of twenty 24-ton feed trucks, all carrying infectious material to different facilities. Simultaneously, the entry causes contamination of the feed manufacturing equipment. In 2017, Schumacher et al. reported that entry of PEDV into a feed mill leads to nearly 100% of surfaces being contaminated, including non-contact surfaces such as walls and floors. Finally, Huss et al. (2017) reported that the cleaning and disinfection necessary to sanitize a virus-contaminated feed mill includes complete organic material removal, followed by wet-cleaning with a glutaraldehyde and later bleach sanitizer. The feed industry is not designed for this type of cleaning and disinfection, so the primary focus must be on keeping pathogenic viruses out of feed mills. This includes a focus on both the source of the ingredients, but also their transportation. The incubation period for ASFV is 5 to 21 days and it may be up to 3 weeks after an animal is exposed before signs of the disease occur. During this time, the feed supply chain may be unknowingly transmitting virus. Therefore, strenuous actions are necessary to prevent feed mills from being a source of cross-contamination.

Recommendations for Swine Feed Manufacturers to Minimize the Potential for ASFV and other FAD Entry and Transmission via the Feed Supply Chain:

1. ***Know your supplier.*** It is key that facilities can identify the supplier of the ingredients coming into their facility. This process helps maintain transparency across the feed supply chain. In some facilities, procurement is independent from quality control and feed safety. These need to be fully integrated, with a system for checks and balances to ensure that the most economical ingredients are used, but only if they are not a potential risk for disease entry. Knowledge of the ingredients supply chain should extend from the point of ingredient manufacture through transportation to the feed mill, including any intermediaries or blending locations.

2. **Do not use grains or oilseeds (or their resultant meals) from regions with foreign animal disease.** Feed mills manufacturing feed for multiple species should follow this for the entire mill, not just exclude it from swine feed. For example, it has been reported that mills manufacturing feed for sow multiplication facilities are also manufacturing organic dairy feed, with imported organic soybean meal from China being used only in dairy feed. Because of the potential for batch-to-batch and environmental contamination, these high-risk ingredients should be excluded from the mill altogether.

3. ***If using other ingredients from regions with foreign animal disease, take steps to ensure they are at low risk for disease transmission.*** The Decision Tree Matrix to Minimize Viral Transmission Risk from Feed Ingredients produced by the Swine Health Information Center and other leading swine organizations can aid in this assessment. In particular, consider both the point of manufacture and its method of transportation. It may be appropriate to have different procedures for receipt of ingredients transported in different forms. For example:
    a. If ingredients are delivered in bulk (vessel, barge, rail, or truck), require washout tickets or proof of low-risk loads since the previous washout prior to receipt.
    b. If ingredients are delivered in bulk tote bag, obtain proof that bags were not reused prior to loading and inspect the bags for damage prior to receipt.
    c. If ingredients transported in small, single-use, sealed bags, sanitize the pallet, pallet jack, trailer floor, and any plastic wrapping prior to receipt. Inspect bags for damage prior to receipt and the use of disallow wooden pallets.

4. **Use porcine-based ingredients with caution.** Porcine-based ingredient production is likely to contain a kill-step capable of destroying viruses. However, post-processing cross-contamination may exist, causing the potential for these ingredients to be sources of viral entry into mills. If porcine-based ingredients are used, obtain these ingredients from suppliers with documented biosecurity procedures and programs to reduce the risk of post-processing cross-contamination. If there is concern about ASFV or CSFV, other animal protein ingredients can be used with negligible risk. If the concern extends to FMDV, ensure that all suppliers of animal proteins have adequate programs to address biosecurity and post-processing contamination.

5. ***Implement biosecurity at the mill.*** Biosecurity procedures have been in place for decades on swine farms to limit disease transmission by people and delivery vehicles. These same principles should be extended to mills.
    a. ***Develop a feed mill biosecurity plan.*** Methods for developing a swine feed mill biosecurity plan are described by Cochrane et al., 2016. Other references, such as the AFIA guide for Developing Biosecurity Practices for Feed & Ingredient Manufacturing, and the K-State Swine Feed Mill Biosecurity Audit are helpful for facilities to determine opportunities for improving biosecurity.

b. ***Use receiving mats or funnels to limit pathogen entry into the receiving pit.*** The ingredient receiving pit is the single biggest entry point for contaminants into the feed manufacturing system. Magossi et al., 2019 reported that the pit was second to only employee shoes as the most unhygienic locations tested in 12 U.S. feed mills.

c. ***Create lines of separation at all doors to minimize contamination from footwear.*** This involves employees and visitors changing shoes to keep exterior shoes on one side of the line and interior shoes on the other. Examples of how facilities may implement lines of separation are shown in Figures 1 and 2. In both examples, additional exits are available in case of emergency to satisfy OSHA requirements. If lines of separation cannot be developed, consider zoning to standardize traffic patterns, with foot baths or food-grade dry sanitizing powder placed in high traffic areas.

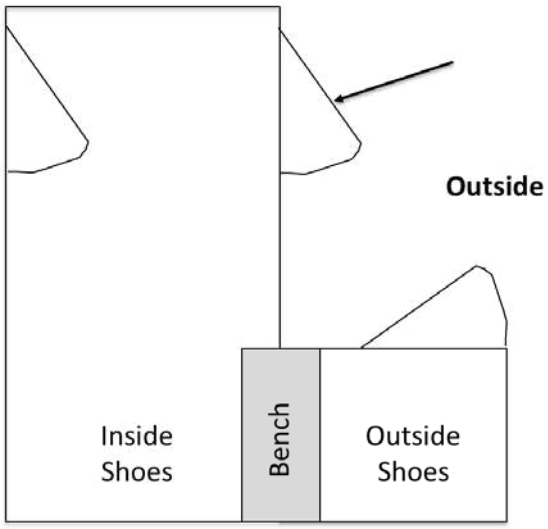

Figure 1. Feed mill entry with a bench delineating areas between outside footwear and that worn inside the mill area.

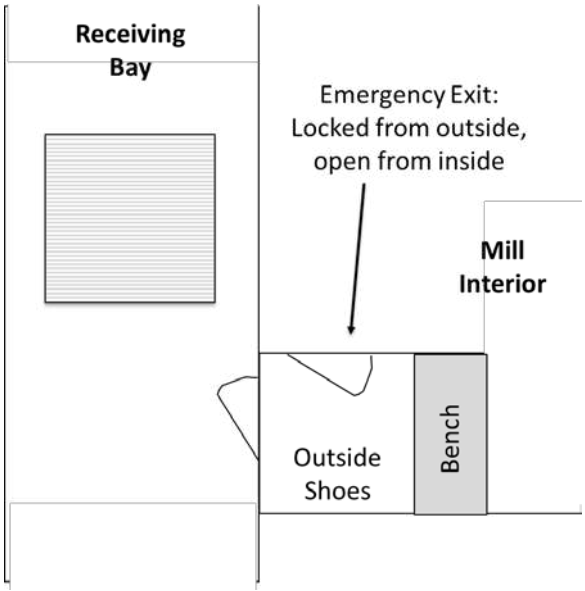

Figure 2. Feed mill entry from the receiving bay delineating areas between outside footwear and that worn inside the mill area.

d. ***Create cleaning and disinfection stations for delivery vehicles and feed trucks.*** Use wet-cleaning and sanitizers to remove debris from the tires, wheels, undercarriage, and exterior of ingredient trucks and feed delivery trucks prior to their entry into the mill. This is particularly pressing in times when disease pressure is high. Be sure that all vehicles are rinsed or dried prior to entry into the mill to prevent cleaners or sanitizers from being a source of contamination themselves.

e. ***Sanitize floors routinely.*** Sweep or vacuum all dirt and dust from floor, then mop on a weekly basis to limit the accumulation and spread of virus on non-feed-contact surfaces. Mopping material should be a bleach solution with at least 2.3% chlorine or an EPA-approved FAD disinfectant, such as a solution with at least 1% Virkon™ S.

f. **Refrain from using dust, screenings, or similar materials as an ingredient or added back into feed production.** These materials are frequently placed back in ground corn or an ingredient bin to minimize shrink. However, dust is consistently reported to carry high levels of pathogens, and should be composted or discarded, never fed to animals.

6. **When delivering feed, use cleaning and disinfection stations prior to entering and exiting farms.** Alternatively, consider unloading feed across a line of segregation or fence into another feed truck or extend bin augers so bins can be filled on the exterior of the line of segregation, as shown in Figure 3.

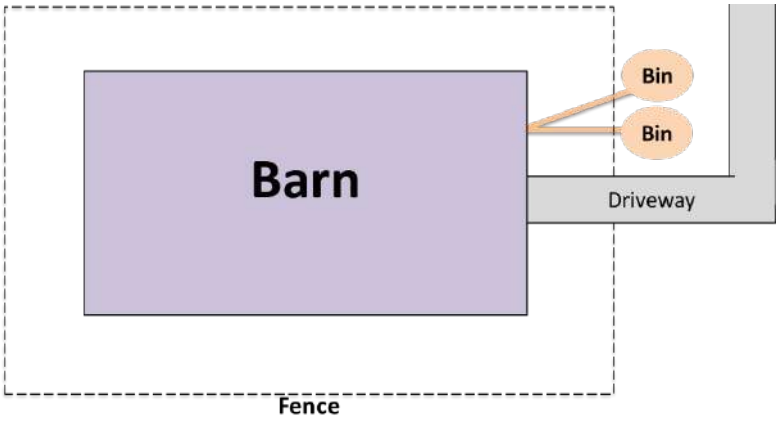

Figure 3. Barn with bins located outside the fenced perimeter so that feed delivery trucks are not required to cross a line of separation to deliver feed.

In our study with the Vietnam production system, a total of 724 environmental samples were collected from the feed manufacturing and delivery process, with 1.1% containing ASFV DNA. One of these positive samples came from a feed mill, with the remainder occurring from feed delivery truck cabs. The positive feed mill sample was from a floor surface where feed delivery truck drivers wear footwear that had been previously exposed to surfaces outside the feed mill. This demonstrates that feed delivery continues to be a weak point in biosecurity protocols, and that footwear must be considered when optimizing feed mill biosecurity.

Conclusions

We are in a new era of feed production, where feed safety is just as paramount as quality and tonnage. Unfortunately, some mills struggle to implement changes that maximize feed safety because it is difficult to establish a Return on Investment calculation for the extra effort. Still, the cost of foreign animal disease entry into a mill would be catastrophic, and therefore we must adapt our culture to make feed that is not just wholesome, but also safe.

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Herd diagnostics and selecting solution for intestinal problems in clinical practice

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Introduction

An important work function for the practicing veterinarian is to solve various problems in swine herds. Diagnostics and making a diagnosis are an important part of solving a problem.

The diagnosis is central for describing the problem, identifying the problem and, finally, making a change that will hopefully lead to the problem being solved.

A diagnosis can either be made for individual animals or, more frequently in swine herds, for a herd – the so-called herd diagnosis. In this connection, it is important to always remember that herd diagnostics are based on the individual animal when the work is carried out.

Type of diagnosis and diagnostic purpose

There are different types of diagnosis. A diagnosis can be clinical, pathological, microbiological and/or based on laboratory tests. There are a number of fundamental principles connected to diagnostics including analytical sensitivity and specificity, diagnostic sensitivity and specificity plus positive and negative predictive values, apparent prevalence and so-called true prevalence. The reader can consult ordinary textbooks on epidemiological subjects in respect of these principles.

Before starting a diagnostic work-up, there are several considerations to be made. For example, how many pigs should be examined, which pigs should be examined and how should they be examined. The first question is what you actually want to achieve – what is the diagnostic purpose? This could e.g. be which infections are causing a cough, is a diarrhoea caused by bacterial infection, how sick are the pigs and what does this cost in respect of productivity, would vaccination be relevant, when would vaccination be relevant or does *Lawsonia intracellularis* have an impact on e.g. growth in this herd.

The choice of the diagnostic method depends entirely on which of these areas one wishes to clarify.

Sample size and selection of pigs

With regard to sample size calculation, it is the case that we should look at how many pigs should be examined. There are a number of statistical formulas for this, but some biological considerations are also involved.

The statistical considerations consist of epidemiological principles which can be found in various textbooks, but in in the real world this often fails as the costs of laboratory diagnostics or practical issues like time available exceed the statistically calculated number. In that case, one must make the best assessment based on practice, economy and statistics.

With regard to the biological considerations in respect of the sample size, it is also important whether it is mixed infections or pure infections. Here it is again a question of what one wishes to examine.

If we look at diarrhoea problems among weaners, we know that mixed infections are a big problem. Here it is therefore necessary to take samples from a quite significant number of pigs in order to uncover which infections are present. On the other hand, if there are problems with an outbreak of pleuropneumonia, then fewer pigs could actually be examined as each sick animal are more likely to represent the problem.

With regard to selection of pigs to examine, there are various methods that can be used: random, convenient, targeted judgement and cluster sampling. Again, this depends a lot on what one wishes to examine, but in the real world cluster sampling and judgment sampling are very often used. Likewise, convenient sampling is often used because the pigs that have died anyway can be used for necropsy.

One should of course always be aware whether the pigs that are examined are in fact representative for the problem one wishes to investigate.

An important thing is also the variation that occurs over time. One should be aware that in respect of pigs we are making diagnostics for the future because we assume that the pigs we examine today are representative for the pigs that will also get diarrhoea or coughing in the future so our findings here will also apply to the pigs in the next batch.

However, we know that this is far from the case. There are great variations between week batches both in respect of pneumonia (e.g. in respect of lung scores at slaughterhouses), the occurrence of intestinal infections in diarrhoea among weaners and finisher pigs and there are other examples.

Diagnostic tests

Finally, there are a number of issues concerning diagnostic tests of which one should be aware. Different methods have different properties in respect of assessing whether an infection is present or not or whether an infection actually has a significance for the disease or pig from which the sample was taken.

For example, we can say that serology is good in respect of determining whether an infection is present in a herd while quantitative PCR is better if one wishes to examine whether an animal is actually sick and has reduced productivity due to an infection. Methods for detection of *L. intracellularis* are good examples of these differences.

There is probably also no denying that histology with detection of agents in histological tissue is the golden standard available for most diseases.

In the future, a change will probably occur in the way diagnostics are done. Among other things, this includes more regular monitoring by using methods that test for many agents at the same time. The taking of samples will also be e.g. with ropes in the form of saliva samples, sock samples as we know them from diarrhoea or samples from air.

In addition, there will probably be progress in pen-site diagnostics which will make it possible for us to make a diagnosis on one animal or a group of animals already on the farm.

Examples of Clinical Cases

Examples in respect of intestinal diseases from practice are e.g. that microbiological tests of post-weaning diarrhoea have shown that Escherichia coli is not always the cause, but on the contrary that rotavirus is the cause or that the diarrhoea is feed-related. It would therefore not be relevant to vaccinate against *E. coli* but on the other hand rather to make a feed-related adjustment.

In the same way, another example concerns weaners and finisher pigs where detection of *L. intracellularis* through normal qualitative PCR or serology does not say anything about whether an infection has an impact on the pigs. Here a quantitative PCR test where the number of bacteria is counted would be able to identify whether there is an impact on the pigs' productivity. In practice, it has turned out that in herds with low excretion numbers of *L. intracellularis* treatment of or vaccination against *L. intracellularis* is not relevant.

Similarly, there are cases of neonatal diarrhoea in newborn piglets, where no agent is detected. This means that it is not a primary, infectious cause but on the other hand e.g. cold or poor milk production of sows. Again examples of how the clinical, pathological and microbiological examination leads to a decision on e.g. not to start up vaccination which would otherwise have been done.

Likewise, continuous monitoring of e.g. PCV2 by quantitative PCR has shown that vaccination is not necessary in all cases. Based on this, it is therefore possible to identify whether a vaccination should be stopped or be continued.

Conclusions

All the things described above apply to the all types of diagnoses and all the different kind of examinations shall be combined for interpretation of the results.

At the end, the most important thing is whether the diagnostics made actually make a difference in respect of handling the problem one wishes to solve.

Attributing *Toxoplasma gondii* infections to sources: current knowledge and addressing data gaps

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Introduction

Toxoplasmosis is an important foodborne disease worldwide. Its public health importance has been largely under-recognized, but recent evidence has shown that *Toxoplasma gondii* leads to a high burden of disease at global, regional and national level. The World Health Organization ranked toxoplasmosis as number 13th among 31 foodborne diseases globally, also demonstrating regional differences, with for example a relative higher importance of toxoplasmosis in the Americas than in Europe (WHO, 2015). Several other studies have estimated a high public health impact in several regions (Torgerson et al., 2015) and specific countries (see. e.g. (Havelaar et al., 2007; Nissen et al., 2017; SCALLAN et al., 2015; Van Lier et al., 2016)).

Humans can be infected by *T. gondii* post-natal (i.e. acquired toxoplasmosis) or vertically (i.e. congenital toxoplasmosis). Because congenital toxoplasmosis is considered particularly problematic due to the severe health effects it can cause in children since birth and the possibility of fetal death, its public health impact has been more extensively studied than acquired toxoplasmosis, which is usually associated with mild flu-like symptoms. However, several newer studies suggest that in some cases ocular disease and severe syndromes such as psychiatric disorders, as well as suicide attempts and traffic accidents, may develop as a result of infection (Burgdorf et al., 2019; Flegr et al., 2014). Furthermore, infections acquired post-natal can cause ocular disease, and atypical strains, which are common in areas outside of Europe, have caused severe toxoplasmosis even in immunocompetent individuals.

Cats and wild felids are the only definite hosts of the parasite, but virtually all warm-blooded animals can act as intermediate hosts, and most species can be carriers of tissue cysts of *T. gondii*. *T. gondii* has been isolated from most livestock species such as pigs, cattle, sheep, poultry, as well as wildlife and game. Like many other foodborne hazards, *T. gondii* can be transmitted to humans through consumption of contaminated foods but also by other routes: through water, soil, or air; by direct contact between people, or by contact between people and animals. The relative importance of exposure from a contaminated environment versus consumption of meat or other foods is still unclear.

To identify and prioritize interventions for reducing the burden of foodborne diseases, evidence on the relative contribution of different sources and routes of transmission of *T. gondii* at regional and national levels is needed.

Source attribution of foodborne diseases

The process of partitioning the human disease burden of a foodborne infection to specific sources is known as *source attribution*, where the term source includes reservoirs (e.g. animal reservoirs like pigs, cattle, pets) and vehicles (e.g. food products like pork or beef) (Pires et al., 2009). Source attribution studies may also be able to distinguish between the contribution of different transmission routes from one or more sources for infection. A variety of methods to attribute foodborne diseases to sources are available, including approaches based on analysis of data of occurrence of the pathogen in sources and humans, epidemiological studies, intervention studies, and expert elicitations. Each of these methods presents advantages and limitations, and the usefulness of each depends on the public health questions being addressed and on the characteristics and distribution of the hazard (Pires, 2013).

Source attribution methods have been extensively used to investigate the contribution of food and animal sources for several infectious diseases, e.g. salmonellosis, campylobacteriosis, and listeriosis. Measuring the proportion of foodborne infections that is attributable to different sources has proven useful in several countries and regions, contributing to *One Health* efforts to guide food-safety interventions based on scientific evidence. However, application of source attribution methods for several zoonotic pathogens is often more challenging, which can be due to the characteristics of the pathogen or due to lack of data. To a large extent, this has been the case of *T. gondii*, for which robust, data-driven source attribution studies in most countries are still lacking.

Overview of studies attributing *T. gondii* infections to sources

Source attribution of toxoplasmosis is particularly challenging due to lack of data, and few studies have been conducted so far. To overcome this challenge, WHO's Initiative to estimate the global burden of foodborne diseases included a large expert elicitation study to assess the contribution of sources for several diseases, including toxoplasmosis. This study estimated that between 42 and 61% of acquired toxoplasmosis cases globally are due to foodborne transmission, with other important routes being water (11-27%) and soil (18-38%) (Hald et al., 2016). The

next step of the source attribution process is to measure the contribution of specific sources within these major transmission routes, which would ideally be based on data on prevalence, contamination and exposure of/to each source.

Opsteegh et al. measured the relative contribution of three meat types for infection with *T. gondii* in the Netherlands (Opsteegh et al., 2011). The authors used a comparative risk assessment approach and concluded that 70% of meat-related infections were due to consumption of beef products, 14% due to sheep meat, and that 11% were attributable to pork products. A more recent study from the same group has improved the model to include the effect of salting on parasite viability, as well as lower concentrations of bradyzoites in cattle, more specific heating profiles, and more recent consumption data (Deng et al., 2020). Results showed that beef remains the most important source of *T. gondii* in the Netherlands, contributing to 84% of the total number of predicted infections in the Dutch population, followed by pork (12%), mutton (3.7%), lamb (0.2%) pork/beef mixed products (0.1%), and veal (0.01%) (Deng et al., 2020). Quantitative risk assessment studies of *T. gondii* in meat products have also been conducted in the UK, Italy, the United States (Condoleo et al., 2018; Crotta et al., 2017; Guo et al., 2017). Guo et al. (2015) performed a qualitative risk assessment of meatborne toxoplasmosis in the United States, and estimated that exposure associated with meats from free-range chickens, and non-confinement-raised pigs, goats, and lamb were higher than those from caged chickens and confinement-raised pigs and cattle (Guo et al., 2015). Belluco et al. (2018) compared the relative risk of *T. gondii* exposure through bovine meat vs pork in Italy, and found that bovine meat was found to be a more likely route of transmission to consumers than pork (Belluco et al., 2018). Condoleo et al. (2018) estimated the risk associated with consumption of different pork products in Italy, and concluded that almost all infections are associated with the consumption of fresh meat cuts and preparations, and only a small percentage is due to fermented sausages/salami (Condoleo et al., 2018). Remaining risk assessment studies developed models that will be useful to inform source attribution models, but looked only into one type of food/ transmission route, and thus do not provide evidence on the relative contribution of sources for infections.

A case-control study in the United States found that the leading foodborne risks associated with toxoplasmosis were eating raw ground beef, rare lamb or processed meats produced and consumed without heat treatment (Jones et al., 2009). More recently, a systematic review and meta-analysis of case-control studies supported these estimates by identifying three risk factors of toxoplasmosis: consumption of raw/ undercooked meat, consumption of raw/undercooked beef, and consumption of raw/undercooked sheep meat (Belluco et al., 2018).

In the absence of data for the application of data-driven methods as described above for a regional and global study, the expert elicitation conducted by the WHO described above also measured the proportion of foodborne *T. gondii* infections attributable to specific foods (Hoffmann et al., 2017). In this study, red meats (i.e. beef, small ruminants' meat and pork) were estimated to cause 50% to 64% of foodborne cases in all regions, but the specific source of that exposure was estimated to vary markedly across sub-regions. Small ruminants' meat was estimated to cause over 40% of foodborne toxoplasmosis in the Eastern Mediterranean Regions, while beef was estimated to cause 30% to 40% in Africa and several sub-regions of the Americas, Europe and Western Pacific. Pork was estimated to account for roughly 20% of foodborne toxoplasmosis in less developed regions of the Americas, and in developed countries within Europe. Vegetables were estimated to play a slightly larger role (21% to 23%) in Europe and South-East Asia than in other sub-regions (14% to 19%). Eggs and dairy are not believed to contribute to foodborne toxoplasmosis.

To our knowledge, these are the published studies on source attribution of toxoplasmosis. Because the geographical differences in the epidemiology of *T. gondii* as well as in consumption habits affect the relative importance of different sources, results from few local studies cannot be extrapolated to other countries.

Main challenges and data gaps

Even though several studies investigated the sero-prevalence of *T. gondii* in different sources, including animals and foods, representative data from all potential sources of the parasite are still lacking globally. Such data are essential for estimating the relative contribution of each source. Furthermore, the variety routes of transmission of the parasite, which include consumption of contaminated meat products, but also other foods, contact with live animals and environmental exposure, make the data requirements and modelling exercises particularly demanding.

The comparative exposure assessment appears to be the best approach to perform source attribution. However, this is a *data-hungry* method, relying on representative data on source contamination, exposure and effect of different processing steps in the survival of the parasite through the transmission chain. The risk assessment studies that have been developed to estimate the risk of disease through consumption of one specific food type can be used as a baseline for further method development. Still to address are substantial data gaps.

What are we doing to address knowledge gaps?

A recently launched pan-European project – "TOXOSOURCES: Toxoplasma gondii sources quantified" - will

collect and analyse data to identify and rank the most important sources of *T. gondii* in the region[1]. In this regional initiative, raw data and published data will be collected for countries across Europe, enabling an estimation of the relative contribution of the different sources for infection. To inform source attribution in the region, TOXOSURCES will estimate the relative contribution of food and environmental transmission routes, provide an overview of the prevalence in food animals and cats, quantify human exposure to possible sources of infection, provide an overview of the processing parameters for relevant meat products, and provide an overview of prevalence and risk factors of human infection. The project will develop a methodological framework and deliver evidence for risk management, including prioritization of food safety strategies. We expect that this approach will be useful derive national and regional source attribution estimates for toxoplasmosis, identify differences between countries, and help understanding the reasons for such differences. Furthermore, the framework may be useful to apply similar studies in other countries and regions.

Conclusions

Conducting source attribution of toxoplasmosis has been challenging globally due to substantial data gaps. This is true even for countries with extensive and well-established surveillance and monitoring of foodborne diseases in foods, animals and humans. The evidence compiled so far points to a high contribution of beef and ruminant meat for foodborne infections globally. Lower attribution proportions have been estimated for pork products by some studies, and raw or undercooked vegetables may also be relevant sources of toxoplasmosis. However, substantial data gaps remain. Importantly, estimating the relative contribution of non-foodborne transmission routes, such as contact with animals or environmental transmission, is crucial to inform public health policies. Secondly, developing and applying more robust data-driven methods, such as comparative exposure assessments and national or regional level, requires collecting representative and comparative data from multiple sources, including source contamination, exposure, survival and dose-response data. We expect that recently launched large scale projects will provide a unique opportunity to address these knowledge gaps, and anticipate that upcoming research will focus on toxoplasmosis and further expand these efforts.

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Selection for resilience in a modern swine breeding program

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Introduction

Resilience was historically ignored in commercial swine breeding programs. One reason was that breeding companies need to supply high health animals to the commercial sector of the swine industry. Nucleus herds needed to be naïve or at least negative for major diseases such as porcine reproductive and respiratory syndrome virus (PRRSv), *Actinobacillus pleuropneumoniae* (APP), *Mycoplasma hyopneumonaie*, and other major diseases. Traits such as mortality have not been added into commercial breeding program selection indices until more recently. Hermesch et al. (2014) estimated that post-weaning mortality should account for roughly 44.5% of the terminal sire index. This represents a major economic value for a trait that has been largely ignored. Post-weaning mortality may be the single largest economic cost due to the cost of the piglet and resources like feed, but many other infectious and non-infectious diseases cause loss of productivity in the commercial sector with causing significant mortality (e.g. heat stress; see Martínez-Miró et al., 2016).

Defining resilience

Many authors have attempted to define and redefine the definition for resilience over the years. Other terms such as robustness sometimes appear with resilience or as a separate definition altogether. Defining resilience and robustness has continued to be a hot topic for authors over the years. Clunies-Ross (1932) first recognized the difference between "resistance to infestation" and "resistance to the effects of infestation". This was the beginning of the separation between 'resistance' and 'resilience', the realization that infestations of parasites or infection by bacteria or virus was not completely correlated to the production of that animal under these diseases.

While studying parasites in sheep, Albers et al. (1987) was very careful to define traits that represented resistance, production (healthy and under challenge), and resilience. Resilience traits were identified by adding 'DEP' to the production trait of interest that stood for 'depression'. This represented the difference (depression) between uninfected and infected environments (i.e. how much production loss occurs for that individual). The loss of production from the uninfected environment is what quantifies resilience in this case.

Confusion has arisen from alternative definitions over the years. Bishop and Woolliams (2014) defined resilience as the productivity of an animal under infection. However, animals differ in their ability to produce under non-limiting conditions (i.e. healthy). Two animals will still perform differently due to alleles making them more suited for the production trait of interest. For instance, melanocortin-4 receptor gene (MC4R) influences feeding behavior and body weight in pigs (Kim et al., 2000). This gene likely influences feed intake regardless of infection status. Therefore, both immune defense and normal productivity are components of production under stressed environments.

Colditz and Hine (2016) chose to separate resilience and robustness altogether. The authors defined resilience as 'the capacity of animals to cope with short-term perturbations in their environment and return rapidly to their pre-challenge status'. Authors contrast this definition to robustness defined as 'the capacity to maintain productivity in a wide range of environments without compromising reproduction, health and wellbeing'. The main contrast being between the macro-level (robustness across environments) to the micro-level (resilience within a certain environment).

To explain resilience, a simple equation can be adapted from van der Waaij et al. (2000).

$$P_c = P_p + f(R)$$

where P_c is the commercial production of the animal, P_p is the production potential of the animal, and f(R) is a function of resilience. This simple equation makes it clear that production under challenge (P_c) and resilience (R) are not the same trait, however it shows there is a clear relationship among the three variables. For example, if an animal is 100% resilient, its commercial productivity is equal to the performance potential of the animal. There is clearly additive genetic variation in resilience (Elgersma et al., 2018; Putz et al., 2019).

To encompass a broad definition of resilience, both infectious disease and non-infectious stressors must be included. Martínez-Miró et al. (2016) reviewed stressors and categorized them into social interactions among pigs, environmental, metabolic, immunological, and human interactions. Heat stress as an environmental stressor has been estimated to cost the US swine industry $299 million per year (St-Pierre et al. 2003). PRRS is estimated to cost US swine producers $664 million annually (Holtkamp et al., 2013). Out of feed events can cause metabolic stress to

animals while growing. Air quality and dust is another common environmental stressor in pigs not commonly studied (Donham, 1990; Senthilselvan et al., 1997). There is no known current estimate of the economic impact of all the above stressors combined, but it is likely in the billions of dollars. Holck et al. (1998) estimated a 30% loss in productivity between a non-limiting environment and stressed commercial conditions.

Although definitions have changed over the years and continue today, too much time should not be spent on defining resilience and robustness. More time needs spent researching and developing genetic evaluation strategies. Commercial data collection schemes should also be evaluated to breed for more resilience pigs in all phases of pork production with a focus on post-weaning survivability.

Selection for uniformity linked to residual variance

Uniformity models have been developed in recent years. This is accomplished by looking into the residual variance of the individual records relative to their contemporary group and additive genetic value. Recently, Iung et al. (2019) published a review summarizing the methods and results of the studies on residual variance (uniformity) to date. Theory for these models has developed in recent years from extensions of the classical quantitative genetics model. Obtaining genetic values for uniformity in a swine breeding program can be accomplished relatively easily because there only needs to be a single record observed such as off-test weight in pigs, making it slightly more practical than other methods (see novel phenotyping below). For instance, some programs are already collecting hot carcass weight in commercial pigs that could be used for this type of evaluation (Dufrasne et al., 2013).

There are several different methods for uniformity models including Bayesian methods (Sorensen and Waagepetersen, 2003), the two-step REML approach (Mulder et al., 2009), and double hierarchical generalized linear models (DHGLM; Rönnegård et al., 2010). Each of these contains two models, one for the mean (typical animal breeding model) and one for the residual variance (with an additive genetic effect embedded within).

To get an understanding of these models, the two-step REML model can be described as follows (Mulder et al., 2009; Iung et al., 2019),

$$\boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{Za} + \boldsymbol{e} \qquad \text{1. Mean Model}$$

$$\mathbf{ln}(\hat{\boldsymbol{e}}^{\boldsymbol{2}}) = \boldsymbol{X_v b_v} + \boldsymbol{Z_v a_v} + \boldsymbol{e_v} \qquad \text{2. Residual Variance Model}$$

where **y** and $\mathbf{ln}(\hat{\boldsymbol{e}}^{\boldsymbol{2}})$ are the response variables for each model, respectively, $\boldsymbol{b}(\boldsymbol{b_v})$ and $\boldsymbol{a}(\boldsymbol{a_v})$ are the vectors of fixed effects and additive genetic (random) effects for each model, respectively, $\mathbf{X}(\boldsymbol{X_v})$ and $\mathbf{Z}(\boldsymbol{Z_v})$ are the coefficient matrices for fixed and additive genetic (random) effects for each model, respectively, and $\mathbf{e}(\boldsymbol{e_v})$ is the random residual effects for each model, respectively. Taking the natural logarithm of the estimated squared residuals makes the distribution closer to a normal distribution. This model has been implemented in the popular mixed model software ASReml (Gilmour et al., 2015).

Variance components have been estimated for these models. The heritability of the residual variance (h_v^2) has a median of 0.012 across 32 studies, most estimates falling within the range of 0.0 to 0.05 (Iung et al., 2019). However, the genetic coefficient of variation of residual variance (GCV_E) has a median estimate across studies of 0.27 (Iung et al., 2019). The GCV_E indicates the potential response to selection for residual variance. Current estimates suggest genetic progress is possible, but likely very slow.

Implementation of selection against residual variation into a breeding program is possible existing implementation into ASReml and MiXBLUP (Mulder et al., 2018). However, with the extremely low heritability estimates, very large family groups would be required. Estimates in aquaculture species tends to be higher and it is possible to obtain large family groups for increased accuracy in these species. Therefore, although it is possible, diverting part of the economic selection index to selection against residual variation using these models in livestock species would be risky for a commercial breeding program, excluding aquaculture species. Furthermore, trials comparing competition in the commercial genetics industry is typically based on the mean, with little to no value typically placed on variation or uniformity even when these traits have economic value (Hubbs et al., 2008).

Precision livestock farming and novel resilience phenotyping

Using technology in agriculture is not new, but one that is a hot topic right now in academia and across different industries (Berckmans, 2017; Koltes et al., 2019). For many years, academia and industry partners have been working on implementing technology into the livestock sector. Examples include individual feed intake stations and ultrasound machines to get carcass measures on live animals. Dairy appears to be leading the way in many respects with other industries not far behind. What seems to separate precision livestock farming (PLF) from normal technology in farms today is the collection of real-time data to make decisions and collect information on farms. Also, most of the technology applied in the past, such as individual feed intake stations, were only implemented on nucleus farms for

breeding companies. Today, PLF has the aim at being implemented at the commercial level as well to help farmers make decisions and track performance of their animals over time. Labor is an increasing issue and finding quality people to be stockmen is a difficult task. PLF has the ability to improve economic viability and increase animal welfare.

There are two main ways to utilize PLF data and technology. Researchers and producers can use the data to 1) collect and monitor real-time data to improve management decisions and catch issues quickly and 2) use the high-throughput data to retrospectively analyze the data to quantify resilience for each individual animal. Both methods are currently under rapid developments in research, although the use of this type of data is not new. Madsen et al. (2005) monitored drinking patterns in a group of pigs in order to detect issues early for management (from a group basis). Today, researchers are finding ways to use video to predict weights in pigs (Fernandes et al., 2018), monitor body temperature (Garrido-Izard et al., 2019), and even detect social interactions between pigs (Chen et al., 2020). Camera vision technology is also being used to quantify activity, eating, and drinking behavior in individual pigs (Mittek et al., 2016). More uses will continue to come up over time, however it will require animal scientists working with other fields, especially computer scientists (Koltes et al., 2019).

The second use of PLF data, retrospectively analyzing the data to quantify each individual animal's level of resilience to obtain novel resilience phenotypes, has just begun. This is a much newer research area and more applicable to breeding programs due to the individual nature of the phenotyping. Novel phenotypes extracted from PLF data could be integrated into a breeding program as indicator traits or breeding objective traits. Elgersma et al. (2018) first quantified dairy cows' resilience by taking the standard deviation in daily milk yield over a lactation as well as quantifying the number of drops in milk yield from several days prior. Daily milk yield was collected using data from robotic milking machines. These resilience traits were genetically correlated to existing health traits in dairy. Poppe et al. (2020) expanded on this research by adding a regression curve of milk yield on day in milk, which should always be done if there are any systematic mean changes over time. Authors found that quantile regression was the best method to extract the residual variation for each individual cow (log of the variance around the regression of daily milk yield on day in milk per cow).

In swine, Putz et al. (2019) quantified resilience in growing pigs using daily feed intake data from individual feed intake stations. Two traits were developed including the root mean square error (RMSE) and quantile regression (QR) phenotypes. For the RMSE phenotype, a simple linear regression was fit for feed intake on age and extracting the RMSE from the regression (see Figure 1 for a simulated example). The QR phenotype was quantified by counting the number of days within animal that were off-feed or below the 5% quantile regression line of all daily feed intake records on age. Both of these phenotypes showed a higher heritability than mortality and were genetically correlated to mortality and treatment rate. These traits are easy to implement into current evaluations as each animal receives one phenotype per time period (e.g. lactation) or lifetime (e.g. juvenile growth). They do not require complex new statistical frameworks like DHGLM models for residual variance that may not be well understood (see above).

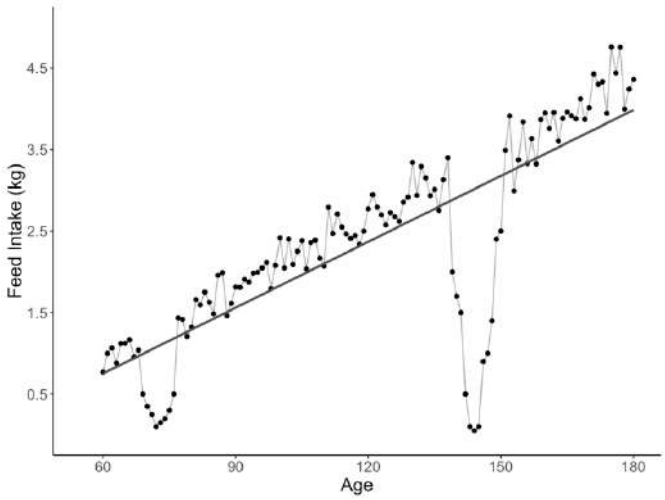

Figure 1. Simulated example of a feed intake curve in a pig with two periods of depressed feed intake due to a stressor such as disease. The regression line can be used to extract the residual mean square error (RMSE) to quantify resilience for each individual animal.

It is important to note that both uniformity models and novel phenotypes from PLF technology quantify general resilience. Although infectious diseases likely cause the most significant economic losses, there are other major losses from heat stress for example. By looking at variation in milk yield or feed intake, it cannot be determined what caused these issues and should therefore be thought of as general resilience to all stressors that impact production traits.

There are many challenges with PLF technology, see Koltes et al. (2019) for a full review on this subject. For

commercial producers looking to use PLF technology to help them manage farms, two main concerns are reliability and return on investment. The cost of some of these technologies can be quite high, especially as many technology companies are just entering this market. For breeding companies (e.g. swine) and national evaluations systems (e.g. Holstein dairy cattle), cost is still a limiting factor, but given the large economic value attached to resilience, the return on investment should be much more attractive, especially if competitors ignore this value.

Inclusion of commercial crossbred data

Improved crossbred performance should be the breeding goal of any breeding company (Hermesch et al., 2015). In the past, companies have relied on or assumed a high, positive additive genetic correlation between purebreds and crossbreds to justify not collecting commercial data. There are also difficulties tracing pedigreed commercial animals back to nucleus animals' reliably and collecting quality data at that level. Estimates of the purebred-crossbred genetic correlation tend to be significantly lower than 1, with 50% of estimates between 0.45 and 0.87 (Wientjes and Calus, 2017). This has lead companies to reconsider collection of crossbred data over the years along with the large economic value attached to resilience (e.g. Hermesch et al., 2014).

Commercial post-weaning mortality data may be the easiest trait to implement into a breeding program. The minimum requirements are 1) single-sire mated F_1 (Landrace x Large White) dams, 2) tagging piglets at birth or weaning to link their pedigree to the nucleus sire (from the central nucleus AI station), and 3) recording individual mortality dates post-weaning. Some companies may want to also have a pedigreed F_1 sow population in order to eliminate confounding between the litter and additive genetic effects of the dam (unpublished results), otherwise a sire model can be fit to the data.

Literature containing commercial post-weaning mortality data are limited as this was not a traditional trait in selection indexes and has not be heavily researched in academic circles. Dufrasne et al. (2015) analyzed commercial mortality data and found the post-weaning survival had a heritability of 0.06, with nursery and finishing mortality showing slightly higher heritability estimates of 0.14 and 0.10, respectively. Authors also found that pre- and postweaning mortality have a low genetic correlation and therefore suggest the control by different sets of genes.

Combined crossbred and purebred selection (CCPS) has been studied over the years (Bijma et al., 2001; Dekkers, 2007). Dekkers (2007) found that adding marker assisted selection (MAS) with crossbred data increased response in crossbred performance 34% over purebred selection. More research is warranted on the advantage of adding crossbred data in the age of computer simulation where more questions can be answered. The issue seems to be the difficulty in programming and computational load to simulate such a large purebred-crossbred breeding scheme and make it flexible enough for any breeding program.

Implications

Stressors in swine cause tremendous losses, with infectious disease leading the way. Management has improved over the years to reduce these losses, however despite these advancements, large losses still persist in the commercial sector. Genetic improvement is one way to make slow but cumulative gain over time to combat losses from stressors. Many commercial genetics suppliers are now collecting commercial data through CCPS systems. Due to the large economic cost to the swine industry, it is important that breeding companies start to focus on commercial productivity by integrating resilience into their breeding goals.

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Management of people in swine production; what data says

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Introduction

During the last decades, the use of data by farmers has been limited. Most of the systems used were simple and mainly focused on the management of farm tasks, with limited or no capacity of analysis. Integration of data from different devices or farms was also difficult, and there was little applied knowledge on the value of data in the strategic decision-making. Another weak point, not solved so far, is the lack of support services in use of data promoting digital transformation and the implementation of systems of information management.

The use of data in many agricultural crops has exploded in recent years; however, its use in livestock is still limited. In pigs, data collection has not changed for many years and analysis is still focused on the main reproductive key performance indicators (KPIs) such as farrowing rate, the number of repeat services, total born, born alive, stillborn, mummifies, weaning to first service interval and preweaning mortality. Other types of data, such as environmental or slaughterhouse data, or data from feeding stations or other automatic sources have not been used in practice except to create simple alerts like detection of temperatures out of range or sows that have not eaten. Among the reasons for this lack of progress are the low added value perceived by producers, the good margins that for years prevented the need for improvement based on production data analysis, the lack of professionals with solid education on-farm data management or the lack of tools to facilitate the process of extracting value, benchmarking and monitoring. On top of these issues, companies manufacturing farm equipment and software that generate data did not facilitate its extraction and use, rather the opposite to protect their equipment and systems.

The Five Steps in New Swine Management System

Data must be transformed into information to generate knowledge. It is not the same despite many times is confused. Companies of any size must set their own information system to solidly support their decision-making process. A swine management information system can be defined as *'A system made up of tools (software and devices) that together with a working protocol and procedures, including the roles of users, can generate the necessary information to diminish the risk and uncertainties in decision-making'*. Such a system always has five steps (Fig. 1), independently of the size and characteristics of the company that uses it:

- **Step 1, Data collection**. Data is the raw material of the system. Must be of quantity and quality enough and can come from human inputs or sensor-robots. Until now, data is just numbers, but the sector is coming closer to the use of images (disease detection based on altered movements patterns, organs and tissue lesions for presumptive disease diagnostics, smaRt Suite™ Ro-main Inc., Quèbec, Canada) and sounds (respiratory distress detected by Sound talks™, Sound talks, Leuven, Belgium).
- **Step 2, Data processing**. Includes several tasks like management of outliers, missing data and use of different formats from different sources. The objective should be the adequate set-up of database structures that allow proper use and interoperability of data (data sharing across systems).
- **Step 3, Reporting**. Deciding and producing the type of reports of interest for the farm or company at every level is not a minor task. From sow cards or working lists (i.e. sows to be mated or vaccinated) up to multivariate regression analysis to define the optimum value for a certain KPI (i.e. age at first mating considering several variables), every farm or company must decide the reports needed by every work level (farm staff, farm manager, veterinarian, technical manager, board of directors or chief executive officers), not forgetting that could be just technical, economical or mixed.
- **Step 4, Distribution of the information**. The objective of this step would be sending the right information to the right person at the right time and using the right channel. This is not properly done in many cases and is an overlooked reason for underuse of data. Sometimes information arrives a bit late and is useless (i.e. hypo-productive sows to be culled if report arrives once mated) or is too complex for farm staff or too simple for veterinarians or managers. User preferences to receive it must be considered as well and can include from classical PDF files, text messages at the smartphone or web applications. Every user will be more comfortable and will make better use of the communication channel is the most adequate.
- **Step 5, Analytics and decision-making**. Information received must be read, understood and used by a person with the right education and with time enough to make a decision to be implemented. Until now, analytics were aimed to be mainly explanatory, but predictive analytics is becoming a key step in most industries due to

the amount of quality data available using artificial intelligence techniques such as machine learning (an application that provides systems the ability to automatically learn and improve from experience without being explicitly programmed) or artificial neural networks (an information processing paradigm that is inspired by the way biological nervous systems, like the brain, process information. It is composed of a large number of highly interconnected processing elements (neurons) working in unison to solve specific problems).

Figure 1. The five steps of an information system.

Every farm or company must follow these five steps to establish a robust information system that supports its production efficiency and required quality standards.

The need for new technologies

In the last decade, the productive global framework is changing. New technologies have been developed in all sectors and are finally reaching livestock farming. Moreover, producers are becoming aware that their competitiveness depends on using their data appropriately to support their decision-making, both for daily decisions as well as strategic ones to improve their competitiveness. The current productive demands are forcing producers to optimise all aspect of the productive chain.

Modern swine genetics demand a higher degree of understanding of their capacities to optimise their performance under commercial conditions. For instance, consider and extend the knowledge in terms of gilts' adaptation period, ages for the first mating, optimization of lifetime performance (Iida et al., 2017), causes of early culling, quality of piglets (small or intrauterine growth-retarded ones), mortalities and mortality patterns (Tani et al. 2017), as well as feeding and feeding patterns (Koketsu et al., 1996a,b) are paramount to get the most of its potential. This information could be fed back automatically to genetic companies and change the current selection structure. It would finally include disease status in the genetic pyramid in an efficient way.

But it is not just about quantity anymore; quality criteria are a key component of competitiveness. Quality must be guaranteed within the production chain of live animals including requirements like piglets with adequate weight, homogeneity suitable for fattening, free of certain diseases or from antibiotics treatments, ensuring welfare status or certain feeding practices, like only vegetables based).

This high demand for good production under high-quality standards cannot be achieved in a production model as complex and sophisticated as the current swine production without adequate use of the information generated.

Biosecurity data

Biosecurity is known as the implementation of measures to reduce the risk of introduction and spread of disease agents (FAO, 2010) and can thus be divided into two aspects. External biosecurity relates to the prevention of pathogens entering a herd, while internal biosecurity prevents the spread of disease within a herd, mainly from older to younger animals (FAO, 2010). In this regard, biosecurity is an important aspect of preventing the transmission of diseases, thus improving health and reducing the need for antimicrobials (Laanen et al., 2013). Moreover, most diseases have a negative impact on the well-being of the animals and consequently, on their productivity. Thereby higher levels of biosecurity lead to also improved the economy of the farmer.

Under our experience performing biosecurity audits in western/eastern Europe and Asia its quite frequent to get the same answer when we find mistakes 'we always do well, but just today…'. And this is one of the crucial points, the exceptions. It is very common that farm staff knows, in general, the theory or the working principles they should

follow, but they don't comply in every case. Following are presented some personal examples:

- **Responsibilities in biosecurity not clearly assigned** to anyone. Some farms have a comprehensive written protocol that is not put in practice because there is no one with the responsibility of its implementation and lack special training on biosecurity.
- **Mixing of personnel in common areas** (canteen) without changing boots or clothes.
- **Shower partially compulsory** because 'you are not going to take them a shower 4 times a day'.
- **'I am special'**. Farm arranged by colours in clothes for every zone but manager wearing special and different ones without respecting rules just because 'I am the manager'.
- **Public and private roads used shared** with no preventive measures at all.
- '**Creativity' in complying with the rules**. 'I only shower when I arrive in the morning'

Data were there wasn`t any

The human factor is known as of paramount importance in swine health and production since most of the tasks are performed by people. This includes how animals are managed in terms of animal care, feeding administration, application in practice of medicines and vaccines, follow-up of biosecurity protocols and accomplishment of farms' tasks and duties plans.

In this scenario of great progress in data management using different sources, our understanding of real human behaviour in swine farms is lacking. Among others, this affects biosecurity and general farms' operations.

The assessment of the biosecurity level of a farm/group of farms is very complex due to the cross-sectional characteristic of the farm. Biosecurity affects all the processes that take place on a farm, animals, people, supplies, environment, etc. For this reason, it is essential to have an evaluation method that is as ordered and standardized as possible, only in this way details that can be of great importance will not be overlooked.

The first and most traditional method are biosecurity audits. These are based on surveys together with a farm visit and a final analysis of data collected. The limitations of this are clearly related to the subjectivity of data reported, sometimes even with the best will, but answers could be more related with a guess than with a fact. When visiting the farm this corresponds normally with the picture of what happens at that moment, which could match totally o partially with a fair image of farm facts and actions performed. Finally, follow-up is mostly based on the repetition of this process rather than in getting objective information about the recommendations agreed. Altogether, biosecurity audits appear at the best possible solution until now to understand and improve biosecurity standards but with a clear margin of improvement.

Despite many diseases share common facts and approach related to the biosecurity principles required, I will focus on one of the most common in the porcine industry; the porcine reproductive and respiratory syndrome (PRRS). This disease impairs swine health and is responsible for huge economic losses in the swine industry worldwide (Neumann et al., 2005). Infection with PRRS virus (PRRSv) is characterized by reproductive failures in pregnant sows, high pre-weaning mortality in piglets infected in utero and respiratory signs in both growers and finishers pigs (Done et al., 1996; Kranker et al., 1998; Rossow, 1998).

Numerous studies (Baysinger et al., 1997; Weigel et al., 2000; Mortensen et al. 2002, Otake et al., 2002a,b, 2004; Firkins & Weigel, 2004; Evans et al., 2008; Dee et al., 2009; Lambert et al., 2012) have described the routes which are involved in PRRSv transmission between and within herds, including the introduction of positive animals or semen, management of the quarantine for the newly introduced animals, as well as vehicles, aerosols, insects or contaminated fomites. Moreover, the marked genetic and antigenic heterogeneity of the virus, combined with its immune evasion strategies, inhibit the full efficacy of current commercial PRRS vaccines (Hu & Zhang, 2014). Therefore, PRRS control based only on the use of vaccination has often provided limited efficacy under field conditions (Geldhof et al., 2013). Hence, it is of paramount importance the implementation of good biosecurity measures to prevent the introduction of the virus into a farm but also to slow down its transmission within a herd once infected.

Nonetheless, most of the developed programs of biosecurity measures are based on scoring systems or survey forms. For instance, researchers from Ghent University developed a scoring system called Biocheck. UGent™ (Laanen et al., 2013; Postma et al., 2016) as a risk-based scoring tool to evaluate the biosecurity quality of pig herds. Another scoring system has been developed by the University of California-Davis (Disease Bioportal®) for the dynamic risk assessment and farms benchmarking also based on surveys (Holtkamp et al., 2013). In this line, Sternberg-Lewerin et al. (2015) developed a risk assessment tool for *B. hyodysenteriae* and *M. hyopneumoniae* considering the frequency of contacts, but it was only focused on external biosecurity. All these tools are based on values obtained through expert opinion panels; however, the perception of experts may vary depending on different circumstances, therefore, scoring systems based on perceptions should be adapted to each situation.

However, the development of objective systems to assess biosecurity is constantly evolving. In this regard, new solutions for digital biosecurity control are appearing in the market as objectives tools for the evaluation of internal biosecurity based on a system of control of the flow of internal movement of personnel on pig farms.

The new digital biosecurity system was based on two on-farm hardware pieces including *beacons* and readers.

Each farm worker was given small *Bluetooth*™ transmitters called *beacons*, which were required to wear all the time while they were within the farm facilities. Readers were installed and fixed at every access of every barn, including lockers and showers. These devices can detect *beacon* signals by proximity. Whenever a *beacon* was within a device's detection range, the device registered the *beacon* identity as well as the detection time and uploads the record to a database.

Data collection and processing records from readers were sent to the cloud and processed, so the movements and routes of the farm's workers were computed. Each movement represented a route made by a farm worker from an origin zone to a destination zone. Thus, the system allowed the real-time monitoring of the farms' staff movements patterns. Figure 2 shows the map of readers set in a farm:

Figure 2. Map of readers to track farm staff movements set in a farm.

Díaz et al. (2020) have recently proved that the internal movements of farm staff are related to PRRVs incidence. First, personal farm movements were classified into Safe, Unsafe and Risk depending on which farm areas are being produced. Results showed that neither the percentage nor the total amount of both Safe and Unsafe movements were significantly different between the PCR Positive and Negative PRRS status groups, being Safe movements was always above 80% in the Negative PCR PRRS status group. However, both the percentage and the total amount of Risky movements were significantly smaller in the PCR negative PRRS status group. These results show a clear relationship between the total amount of Risky movements and the probability of a PRRSv outbreak on the farms (Fig. 3).

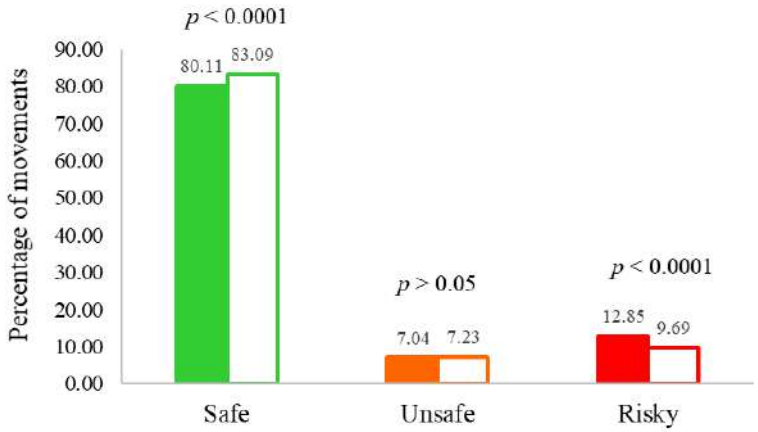

Figure 3. Average of percentages of Safe (green), Unsafe (orange) and Risky (red) movements before (■) and after (□) the training session considering the eight farms in which the system control movement was installed.

Moreover, proper training can decrease risky movements and increase the safe one (Fig. 4).

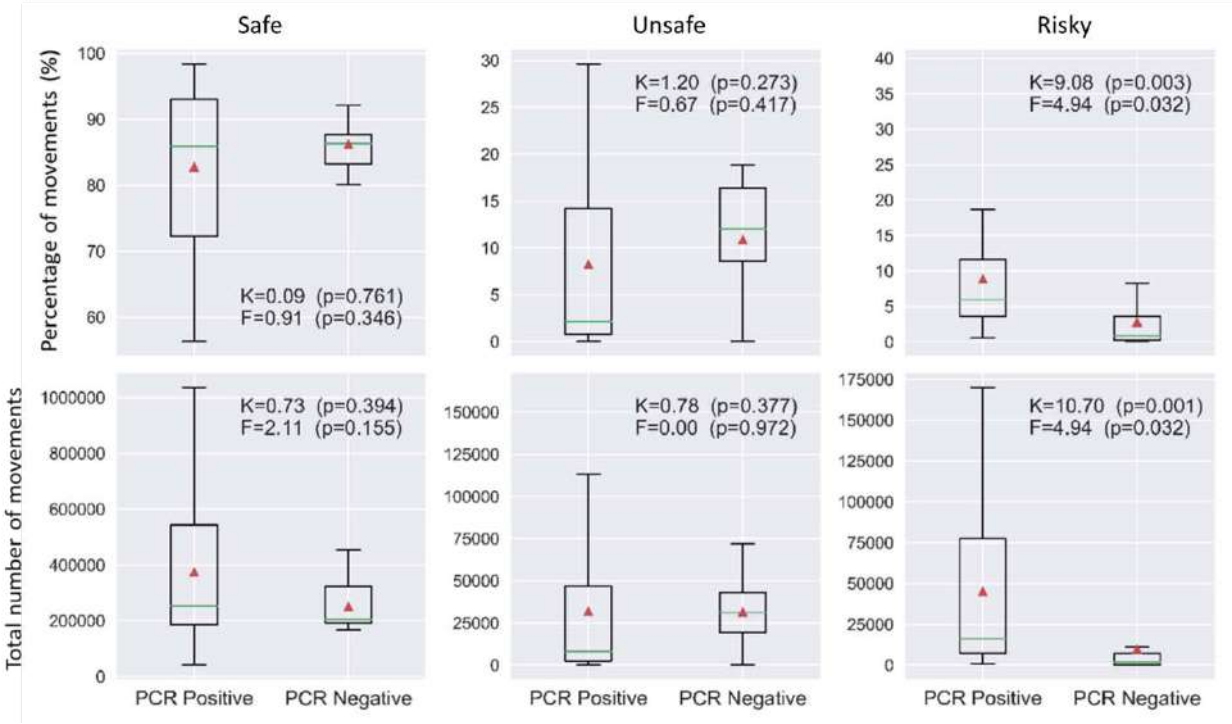

Figure 4. Comparison of percentages and totals of Safe, Unsafe and Risky movements between the PCR analytics positive and negative groups. The boxes extend between the first and third quartiles of the data for each group, and the whiskers extend between the minimum and maximum values. Group average is shown by the red triangles, while median lines are shown with solid green lines.

Operational data

Farms' operations must be performed every day more with a higher degree of accuracy to ensure expected results. Generating the expected number of pigs of a certain quality is a consequence of a good number of ordered actions adequately performed. Most of those tasks are performed by people and, generally, little is known about how it is really performed beyond of reports or checklists normally filled by farm staff or managers despite is not common yet in pig production, some companies are starting to use some techniques coming from other sectors to meet these objectives, including LEAN methodology, Six Sigma, Kaizen, Hoshin Planning or Balanced Scorecard in order to meet operational excellence. This can be described as a philosophy that embraces problem-solving and leadership as the key to continuous improvement. People are often unsure of how to approach the subject of operational excellence. It is a difficult term to define and most people either find the topic to be too ambiguous or too broad to talk about. Operational excellence, however, is not a set of activities that you perform. It is more of a mindset that should be present within you and your employees. Now, you're probably thinking, "that sounds nice in theory, but how do I translate this into actionable steps?".

These technologies allow moving forward into that direction in an objective way, understanding better how human behavior influences farms' results. Recent work from Arruda demonstrated a relationship between the movements and the efficiency in production in US farms (data not published). In her study, a five-hour increase in time spent in rooms by the manager was associated with an increase in one weaned piglet for every 10 litters (P = 0.01). In a second farm, an increase in time spent working in the farrowing rooms of approximately two hours per worker per week tended to increase the number of weaned piglets by one piglet for every 4 litters (P = 0.087). These findings could be related to an increase in pig care, which would likely lead to a higher number of weaned animals. Having control of this at a glance will be of great help to monitor that operations are performed as expected and easen early personalized corections (Fig. 5).

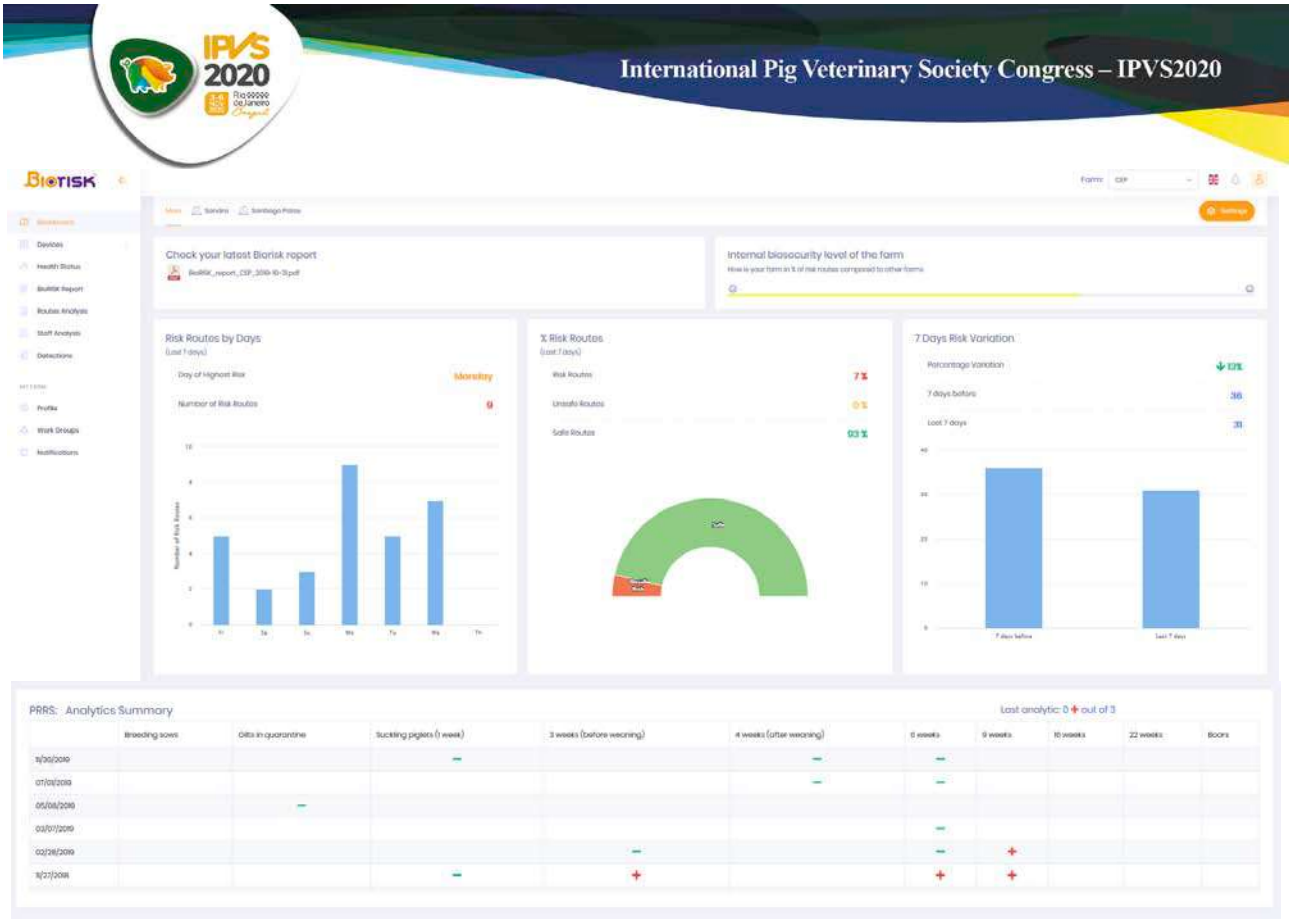

Figure 5. Key biosecurity indicators monitored real-time in a farm, including lab results.

Under our own experience, we have observed in farms worldwide some interesting facts that relate human behaviour and farm performance, both from visitors and farm staff:

1. **Football World Cup 2018**. In some farms, it was observed how on the days of national team games, the time spent by the workers in the mating area was reduced up to 35%.
2. **Repeats of gilts**. In a Central America farm, we detected an unusually high percentage of repeats in gilts during the weekend. The time people spent in the mating area during the weekend was 40% lower compared to the rest of the days and the multiparous area.
3. **Gilts development unit**. Wrong and forbidden farm staff entries during the quarantine period to gilts development units barn were detected which lead to immediate preventive or corrective actions. Remarkably, nothing was informed from farm staff about these facts.
4. **Lactation feeding management**. When feeding sows manually in lactation it is recommended to space out the feed delivery to promote higher intake and less wastage. We have found evidence that the third feed delivery, expected in the afternoon, was brought backwards considerably during the weekends, to finish the job earlier.
5. **Holidays**. In Spain, we analyzed the movement and entry-exit patterns during the stay in certain areas of the farm. When one of the workers were on holiday and another covered the position, the pattern changed and the stays in that particular area were shorter and the entry-exit was more frequent. We believe that, due to the lack of habit of working in the area, the performance of the task was less efficient.
6. **Facilities**. In Spain, we also evidenced that the workers of the farrowing area spent more time in old barns to wean the same number of piglets that in new barns.

Metrics focused on hours spent by farm zone or by farm staff can be very useful to really understand the work performed and its pattern (Fig. 6).

Finally, it must be mentioned the use of these digital technologies to control external biosecurity, mainly related to access control of visitors, trucks and company staff. Some companies in Latino America has set a system to control the access of the staff to farms, since in large production systems, along with the transport of animals, it is considered one of the greatest health risks of entry of diseases. The first company that implemented the system controlled almost 400,000 accesses of their workers and contractors in the first year (Figures 7 and 8).

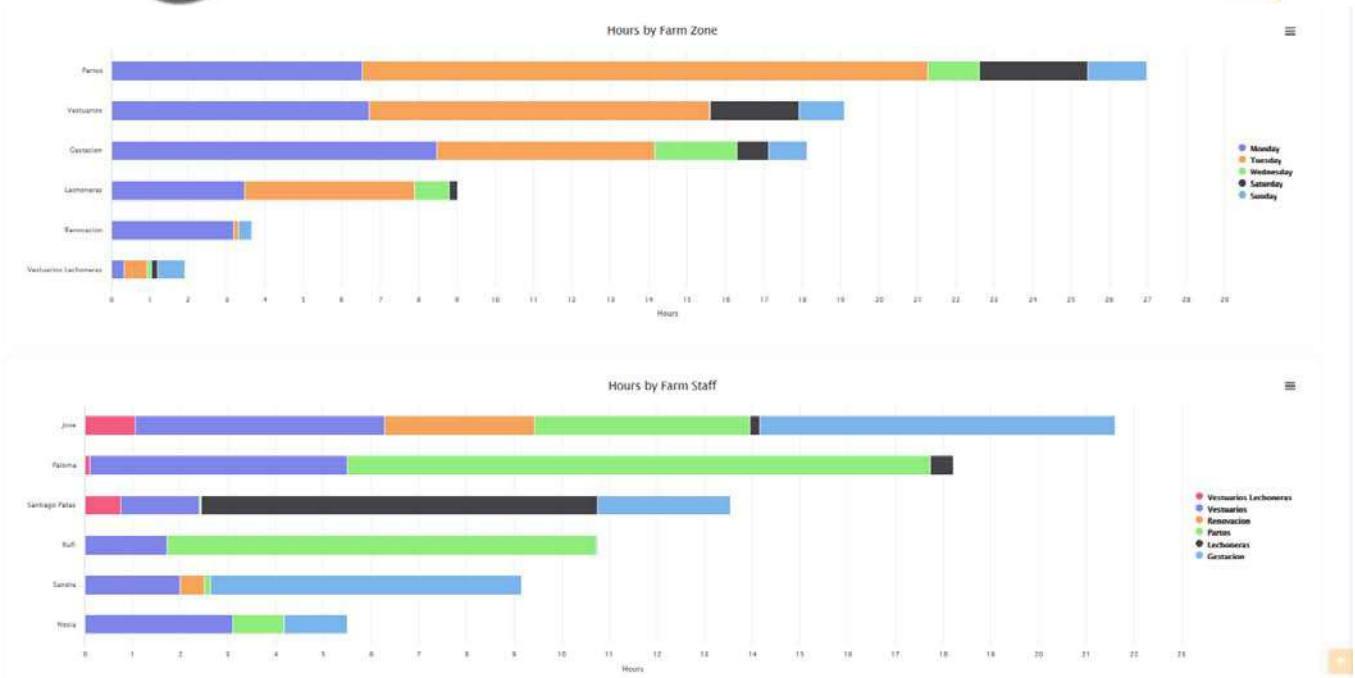

Figure 6. Charts showing hours spent by zone or by farm staff in a farrow to wean farm monitored real-time.

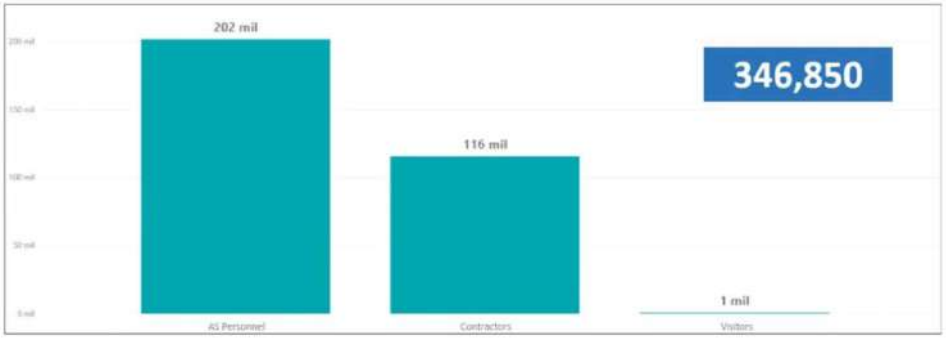

Figure 7. Number of visitors controlled in the system. October 2018 – June 2019.

Figure 8. Overtime entries and failures detected by the system. October- December 2018.

If every entry is a risk, it can be said that large companies, including this, are exposed to extreme risk. The system allowed to effectively control the flow of workers in farms responding to the restrictions of biosecurity established according to the company criteria, providing at the same time absolute traceability of all movements made

and the possibility of immediate response. In this way, and in addition to the entry control, objective risk indicators are generated to manage and ensure biosecurity standards set. These new indicators are called "key biosecurity indicators" (KBI) and allow to keep an online and instantly updated record in the cloud (unlike traditional paper systems which are difficult to understand and generates slow reactions). Figures 9 and 10 show some risks of entry:

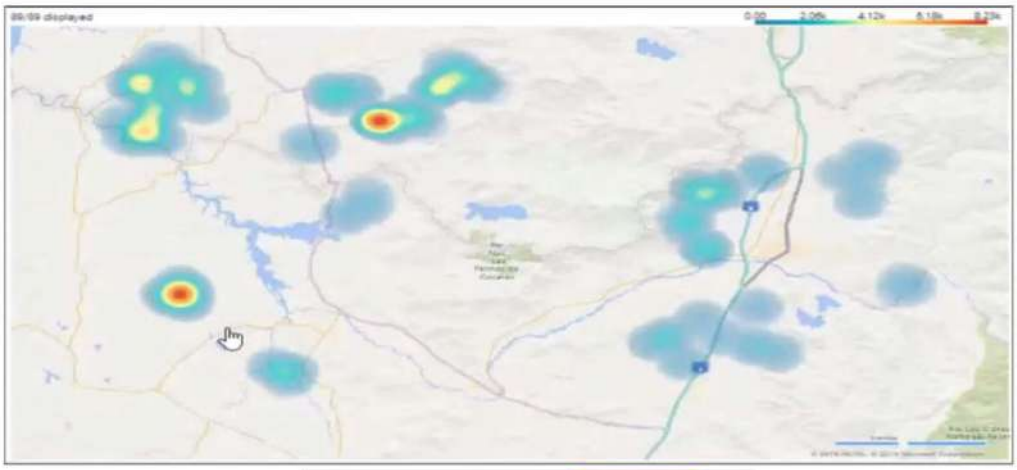

Figure 9. Total number of entries per farm.

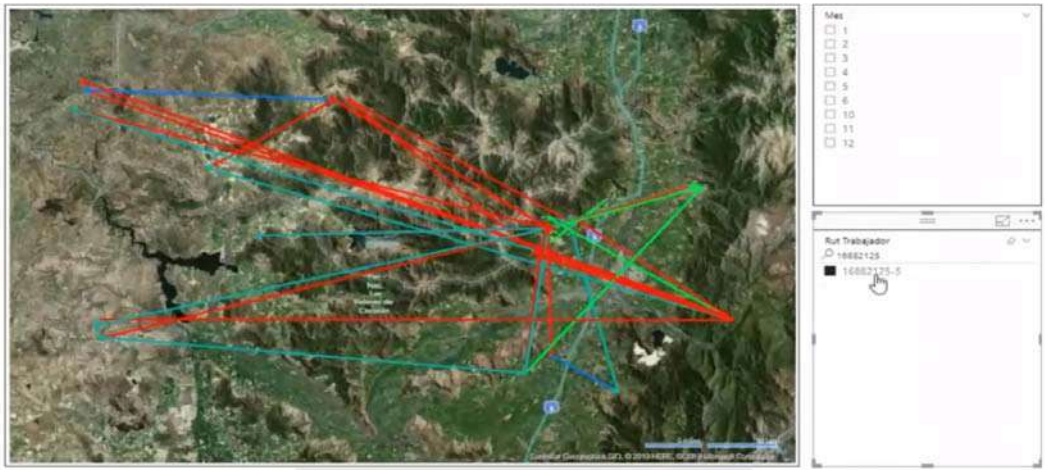

Figure 10. Traceability of movements among farms.

The system allows generating:

- Instant alerts to minimize damages, early control of its extent and prompt corrective actions.
- Easy monitoring of compliance with company standards.
- Detect those factors that generate more risk to the system and work specifically in its correction.
- Puts pressure on respect for rules since behaviour improves where users are aware of being controlled.
- Perform biosecurity audits more objective, effective and focused since they are based in data and not in checklists
- Design tailor-made training programs based on errors and not using the generic ones.

Conclusions

Swine production sector is becoming more professional than ever since it will be the only way to meet the objectives of efficiency and quality that support its competitiveness. Among other challenges, ensure high biosecurity standards to keep farms free of disease and therefore improve productive efficiency and quality standards, mainly related with a low or minimum use of antibiotics will be one of the key most important challenges in the next decade. Besides of this, promoting good or excellent operations will greatly help to achieve the objectives mentioned.

Digital technologies, are very reliable and cost effective will be a great new tool for the sector and in particular for veterinarians that can do more and better consultancy work, without the mandatory need of visiting the farm, being able

to understand better many factors that influences health and farm performance based on staff behavior, developing better solutions and deliver customized training to specific workers based on their behavior ad performance.

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Viral Disease in Swine: An Immunologist's Perspective with Focus on PRRS

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Introduction

The controversy that disease was transmitted by invisible life forms stretches back centuries dating to Aristotle (300BC) who believed that all life originated from soil and Virgil (40BC) who believed that bees came from honey and maggots originated from warm meat. It was Spallanzani who showed that no maggots arose from boiled meat while others showed that simple cotton filters could prevent spontaneous generation of life from warm meat. The idea that invisible substances caused disease was proposed by Varro already in the second century BC, but a millennium passed before Fracatorius proposed that syphilis was one of these invisible "germs". Finally, Leeuwenhoek in the 17th century invented the microscope and provided the first evidence that these potential "germs" were actually visible. In the 150 years that followed, many showed that some of these microscopic forms were responsible for disease. However, it would be Iwanowski in 1892, who showed that substances not removed by bacterial filters could still cause disease and especially noteworthy was that these small "viruses" could not be grown in free culture like bacteria but required a cell source; alas, viruses must be cell parasites. Consistent with the chronology of discovery, it is not surprising that the first contagious diseases characterized were caused by bacteria, the organisms held back by the cotton filters. While the last portion of the 19th century and the beginning of the 20th century was dominated by the identification of bacteria-borne diseases, the last half of the 20th and the 21th century witnessed the regular discovery of diseases caused by viruses. As the rate of their discovery increased, it became known as the era of emerging viral disease.

In humans, HIV, SARS, MERS, Ebola, Marburg, Lyssa, Dengue, Hendra, Nipah and new coronaviruses emerged. Neurotropic paramyxoviruses like Japanese encephalitis viruses (JEV) and West Nile (WNV) had devastating effects. Since its appearance in 1999, WNV has caused >300 deaths in the US, and more than 50,000 have been ill from JEV, of which half were in China. Add to this the ~ 500 million cases of Dengue reported worldwide and the rise in enterovirus infections (Wilson 2013). Some human viral diseases are zoonotic and infect the species around which this International Congress revolves (Tab. 1). These include influenza A, Nipah and SARS. Nipah has led to hundreds of deaths and the slaughter of a million pigs (Sezzad et al., 2013). In a study by VanderWaal and Deen (2018) based on articles cited in PubMed, seven of the top 10 diseases of swine are of viral origin, although enteric bacteria (*Salmonella* and *E. coli*) still topped the list (Fig. 1). We did a PubMed survey for 2019 and found a major shift in apparent importance. PRRSV previously in sixth place, vaulted to first place for all swine disease, with PCV-2 close behind and Nipah moved from 37th to sixth. Figure 1 is a reminder that the once invisible and non-filtrable life form we call a virus, has become the dominant pathogen in 70% of the major diseases of swine. Figure 1 also shows that PRRSV and PCV-2 replaced E. *coli* and *Salmonella* at the top of the list. This shift illustrates that veterinary microbiology is now more concerned with viral disease than enterobacteria. Table 1 summarizes the major swine viruses, emphasizing their economic impact, whether they have zoonotic potential and whether vaccines are available (Lager and Buckley, 2019).

Table 1: Viral Pathogens of Swine

Virus	Economic Importance	Vaccine	Zoonotic Potential
ASF	++++	No	No
FMD	++++	Yes*	No
CSF	+++	Yes	No
ADV	++	Yes	No
PRRS	++++	Yes*	No
SIV	++	Yes	++++
PCV-2	+	Yes	No
PED	+	Yes	No
Seneca	+	No	No
Japanese encephalitis	+	Yes	++
Hepatitis E	+	No	++
Nipah	+	No	+++
Encephalomyocarditis	+	No	+
Menangle	+	No	+
Vesicular stomatitis	+	No	+
Vesicular exanthema	+	No	+

Data assembled by Lager and Buckley
*= maybe strain-specific

Another virus for which no vaccine exists, and which is receiving more attention is Nipah, which like influenza A, is especially zoonotic and uses fruit bats as a major vector (Fig. 1 and Table 1). Influenza A especially affects humans and swine, and some regard swine as the "mixing bowel" for the influenza A genome (Urbaniak and Markowska-Daniel, 2014). Treating influenza A in humans, requires that the CDC decides each year on a killed vaccine that their prediction models suggest will provided protection against the variants expected to be present in a particular year. Currently, much effort is underway to produce a truly universal polyvalent vaccine that might result in some unemployment at the CDC but would also result in a world safer from influenza A.

In the last half century, inefficient family farm swine production has been replaced by industrial scale farming which presents challenges in the control of especially emerging viral diseases. While improved security and use of antibiotics can often control enteric bacterial pathogens, there is no universal pharmaceutical control of viral infection, which depends on the host immune system, both active and passive. In the absence of effective vaccines, diseases like ASF can only be controlled by euthanization of infected herds and destruction of their carcasses. ASF was for a long period confined to regions of Africa, with periodic and controlled outbreaks in Spain and Portugal in 1957-1960. Sadly, ASF spread to the Caucasus and surrounding region in 2007 resulting in the destruction of >140,000 animals in Romania and >800,000 in that general region of Europe (Sanchez-Codon et al., 2018). ASF emerged in 2018 in China and has disseminated one-third of the swine population of that country (Lu et al., 2019). Without an effective vaccine, ASF remains a disease out of control. FMD is one of the longest studied animal viruses and its control history follows a similar path as now being used to control ASF. While vaccines are available, they tend to be serotype specific and lack cross protection for the seven known FMD variants (Mahapatra and Pandi, 2018). While there are a number of PRRS vaccines, full efficacy has not been obtained and like FMD, are often serotype specific, presumably because of the enormous strain diversity of this RNA virus. Currently > 30,000 variants are known but only a small number have been tested *in vitro* or *in vivo* for their virulence (Faaberg et al., 2012). The danger posed by the genetic variability of PRRSV is exacerbated by the ability of this viruses to modulate the host's immune system. This is the major focus of this article.

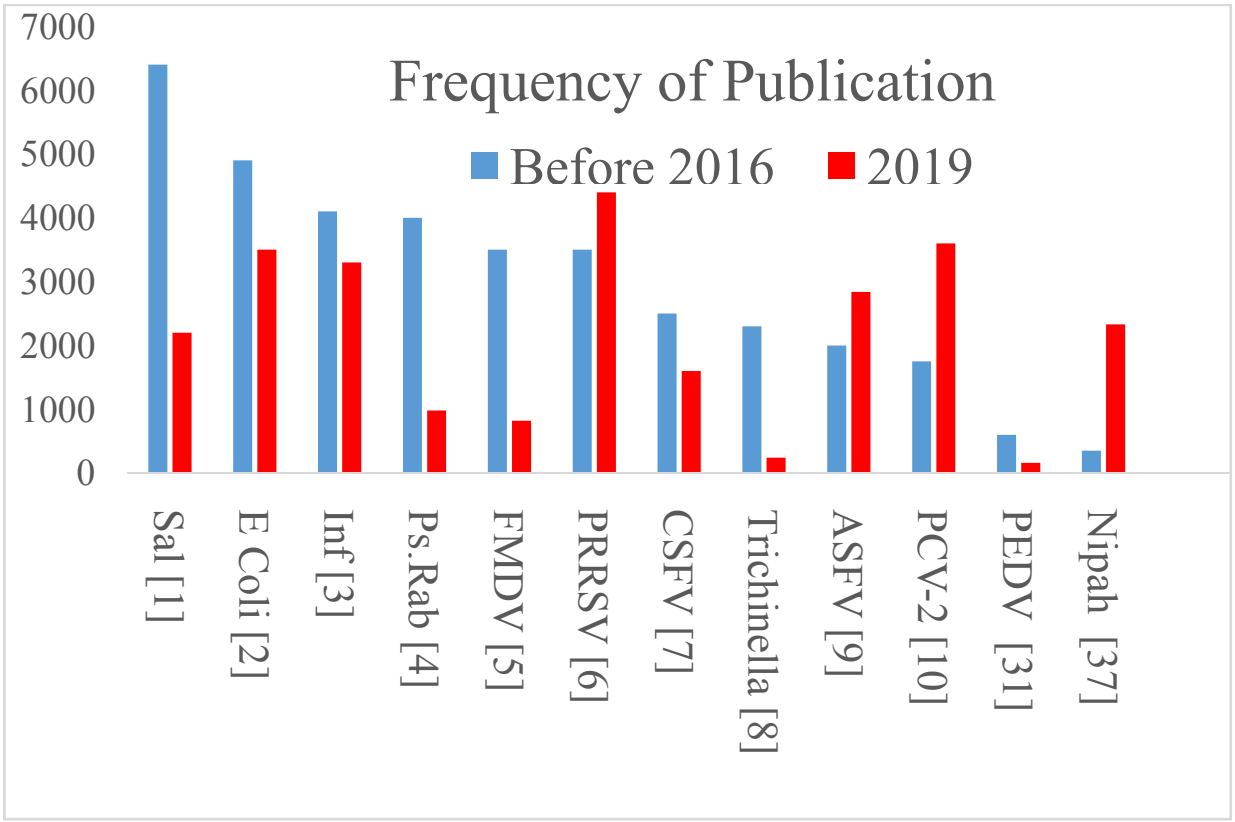


Figure 1: Frequency of publications in PubMed on diseases in swine from 1996-2016 (blue) and the frequency of publication cited in PubMed in 2019 (red). In order to use a single Y-axis, values for 2019 were inflated 20-fold. Numbers in parenthesis for each pathogen indicates their rank in the 1996-2016 study

The Critical Window of Immunological Development

Mammals are born with a functional cardiovascular, digestive, and skeletal-muscle systems but their immune system must develop during the period we call the Critical Window of Immunological Development (Butler et al., 2006; 2014). Mammals like swine are born with only an innate immune system, and receptors capable of distinguishing the biomolecules made by prokaryotes, i.e. bacteria and viruses, from those common among vertebrates and multicellular eukaryotes. Best known among the innate receptors that recognize prokaryotic biochemistry, are the Toll-like receptors (TLRs; Beutler and Rietschel 2003; Giradin et al., 2003). *Toll*, being a German word for beautiful was used to describe them when first encountered in fruit flies (Hoffmann, 1995). Table 2 summarizes the major TLRs and their specificities. In contrast to innate immunity, newborn mammals inherit only the rudimentary building blocks for developing their adaptive immune system that must be somatically generated and cannot be passed on to their offspring (Fig. 2). Adaptive immunity can be considered as the evolutionary response of higher vertebrates to the high rate of genomic change capable in bacteria and viruses (Table 3). We call this "*The response of the Jedi Knight Lymphocytes to the Microbial Empire"*.

Table 2: Specificity of Some Common Toll-like Receptors

TLR	Ligand	Common Target Microbe
TLR1	Triacyl lipopeptides	Mycobacteria
TLR2	Peptidoglycans	Gram-positive bacteria
	Lipoproteins	Mycobacteria
	Zymosan	Yeast & fungi
TLR3	ds-RNA	Viruses
TLR4	LPS	Gram-negative bacteria
	F-protein	RSV
TLR5	Flagellin	Bacteria
TLR6	Diacyl lipopeptides	Mycobacteria
TLR7	ssRNA	Viruses
TLR8	ssRNA	Viruses
TLR9	CpG oligonucleotides	Bacteria DNA
.	Herpes infection	Some herpesviruses

Table 3: The Microbial Empire versus the Jedi Lymphocytes

Rate of genome change assuming a nearly constant mutation rate equals:
Genome size x Reproductive rate
Humans = 8.6 x 10-12
Bacteria = 1.2 x 10-9
Influenza = 2.6 x 10-5

Thus:
Bacteria can change > 100 fold faster than humans
Viruses can change > 1000 fold faster than humans

Lymphocytes of the adaptive immune system can through somatic gene recombination generate > 10 6 variants than can be increase to >109 variants by somatic mutation

Thus:
The Jedi Lymphocytes can more than compete with the rate of genomic change of microbes

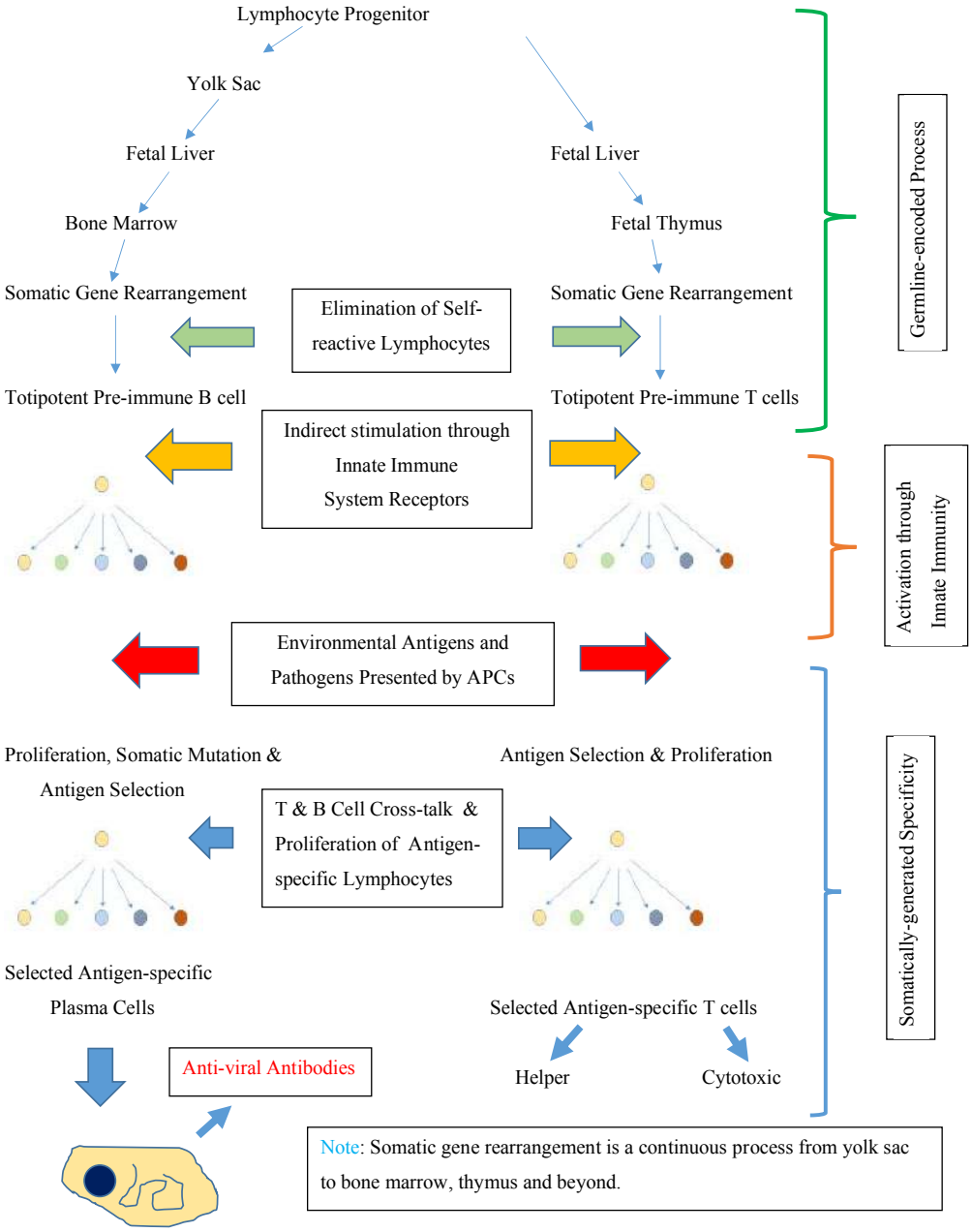
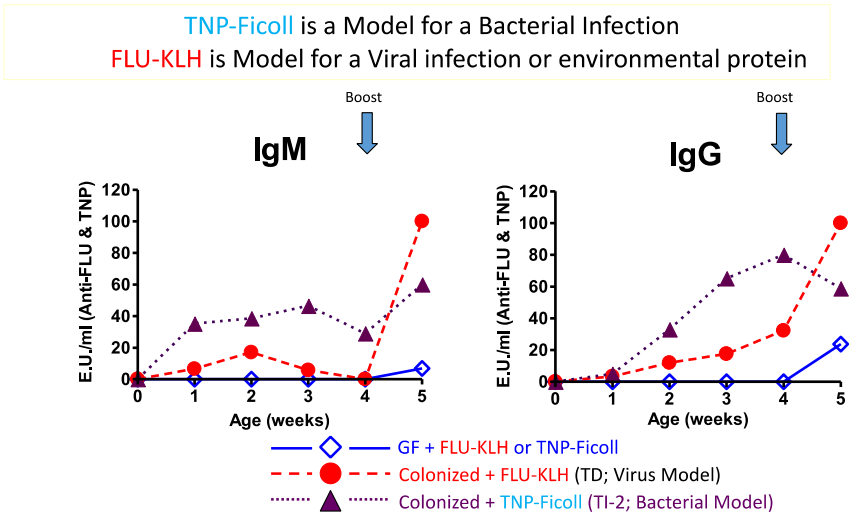


Figure 2. Ontogeny of lymphocytes development from progenitor cells to virus-specific B and T cells. Pathways include those genomes encoded (green brackets), events stimulated by encounter with receptors of the innate immune system (orange brackets) and events that are somatically generated and cannot be passed to the offspring (blue brackets). [Reference Table 3]

The development of adaptive immunity in mammals, in particular in swine, requires colonization of the gastrointestinal tract (GIT) by bacteria or viruses or exposure to ligands recognized by the TLRs (Butler et al., 2002, 2005; Fig.3). This exposure triggers the somatic processes that lead to development of specific and effective protective VN antibodies (Fig.2). Development of T- and B cell repertoires in swine starts *in utero* (Fig. 2). B cell development begins in the fetal yolk sac, moves to the fetal liver and continues for life in the bone marrow and through the process of somatic gene recombination, an array of B cells with their B cell receptors (BCRs), generates a pre-immune repertoire (Sinkora and Butler 2016: Fig. 2). Meanwhile, the same occurs in the fetal thymus which generates a corresponding pre-immune T cell repertoire (Fig.2). Development proceeds so that only B cells and especially T cell populations that do not recognize self-antigens, are allowed to survive (Fig.4). It is these surviving B- and T cells populations that are

charged with the responsibility of recognizing foreign antigens in newborns. Viruses able to gain access to the fetus, such as PRRSV, parvovirus and Nipah virus, would theoretically be recognized as "self", and thymocytes recognizing them would be deleted, thus after birth, the neonates would be "blind" to any infectious agent that had thrived in the fetus. Fortunately, the healthy fetus is normally sterile, so tolerance to pathogens does not normally develop.



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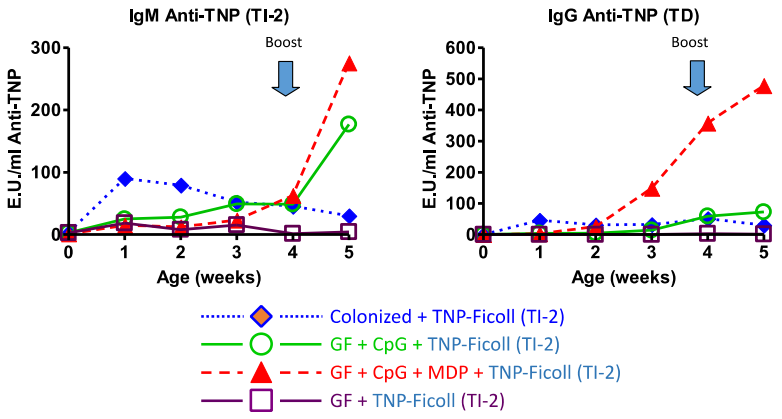

B

Figure 3. A. Bacterial colonization is necessary to allow isolator piglets to develop adaptive immune responses to model bacterial and viral antigens. B. TLR ligands alone enable germfree isolator piglets to make IgM and IgG antibody responses. MDP=muramyl dipeptide, a ligand for TLR 2 and CpG, a ligand for TLR 9. (Reference Table 2).

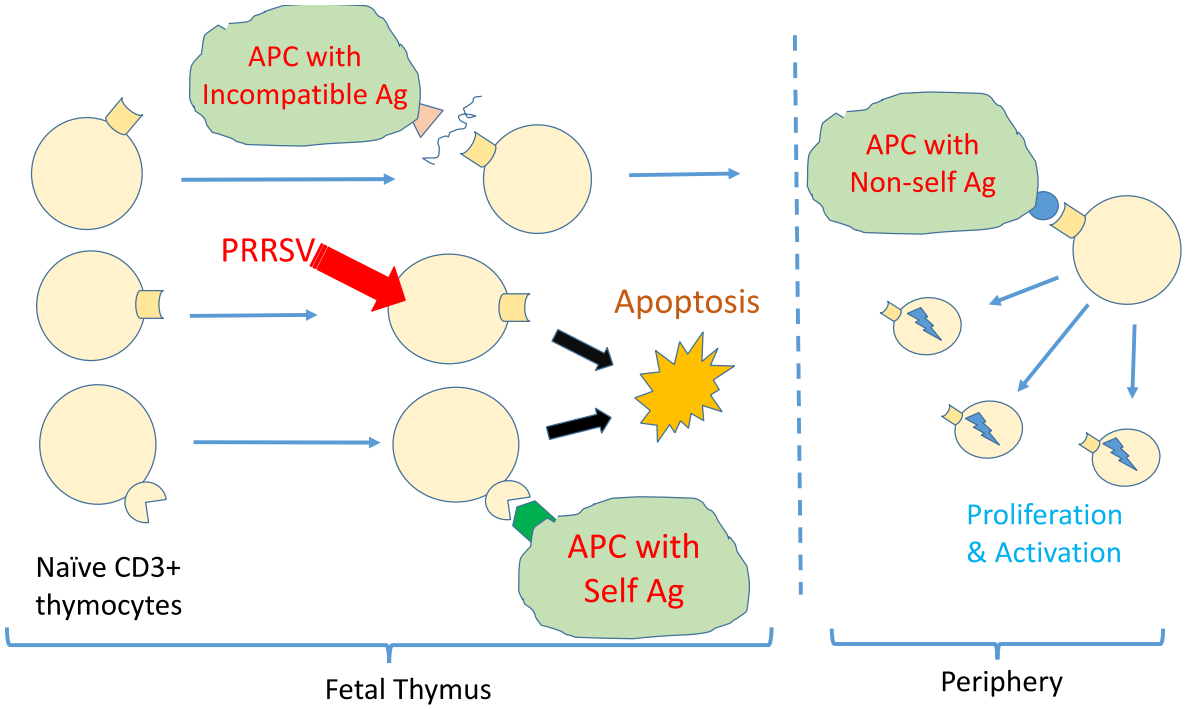

Figure 4. Illustration showing that developing thymocytes that are infected with PRRSV, are eliminated thus reducing the size of the peripheral T cell repertoire. Also illustrated is the apoptosis of developing thymocytes that recognize self-antigens. In infected fetuses, the antigens of pathogens would be considered as self, thus removing potential pathogen-specific T cells that emerge and populate the periphery.

Developing offspring also benefit from passive adaptive immunity. In humans this begins during fetal life by *in utero* transfer of maternal antibodies. In swine and other Artiodactyls and Perrisodactyls, this transfer of passive antibodies occurs only after birth via colostrum and milk (Fig.5).

All of the events described are necessary for the adaptive immune system to develop and these occur within a concentrated neonatal "window of life" that stretches from conception to weaning in piglets and to puberty in humans. Within this window, the highest frequency of mortality and morbidity occurs for offspring of all multicellular life forms including invertebrates, oak trees and flowering plants. This is also the period when infectious disease claims the offspring of vertebrates as their victims. We have called this the Critical Window of Development (Butler et al., 2006; 2014).

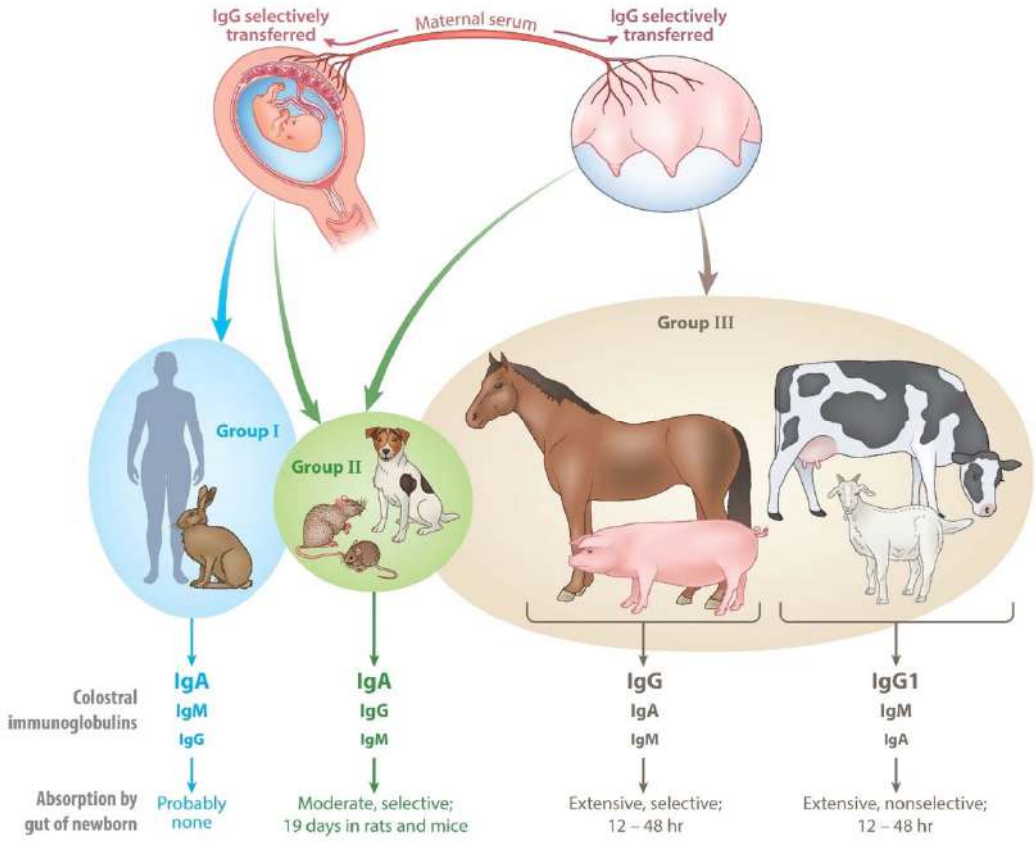

Figure 5. Transmission of passive immunity from mother to young among common mammals. The size of the symbols for the different immunoglobulins, e.g. IgA, IgG etc., indicates their relative levels in colostrum. From Butler 1971, Butler et al., 2017.

PRRS: A Porcine Pandemic

Porcine reproductive and respiratory syndrome is a pandemic caused by an Arterivirus (PRRSV) that affects all life stages, but especially animals in the Critical Window of Development. Some estimate that PRRSV is responsible for a yearly $600,000 lost to the US pork industry (Holtkamp et al., 2013). The syndrome was recognized in the 1980s and the virus identified in 1991 (Collins et al., 1992; Wensvoort et al., 1991). Since then >3,000 articles have been published on this disease it has become the major studied disease of swine (Fig.1).

PRRSV is one of several known viruses to cross the placenta that includes parvovirus and Nipah. Infected fetuses suffer from vasculitis, especially of the umbilicus which could be the cause of their death. Fetal death is not uncommon in swine, but it is not a cause of abortion. However, infection of the sow can cause abortion "storms" even if the litter is not affected, possibly through some neuro-endocrine mechanism. Since PRRSV is highly contagious, an infected sow can readily infect her healthy newborn litter causing a respiratory disease that increases the risk of secondary infection (Done and Paton 1995; Van Reeth et al., 1996, Renukaradhya et al., 2010, reviewed in Butler et al., 2014). Altogether these pathologies explain the origin of the name PRRS.

The strategy for many viruses is to modulate or dysregulate the host's immune system, just enough to delay the anti-viral response and expulsion of the parasite, but not by killing the host; "*Good parasites do no kill their hosts*". The first response of the infected host is the responsibility of the innate immune system, through recognition by TLRs, which stimulates the production of type I interferons (IFNs) which induces resistance to viral infection by inhibiting replication of the viral genome and by up-regulating MHC I expression on infected cells. This enhances the ability to display viral antigens that can be recognized by T and B cell receptors, which triggers the engagement of the adaptive immune system (Fig. 2 and 3). Suppressing MHC I expression on infected cells is a common tactic used by viruses to prevent the immune surveillance system from recognizing a virus-infected cell. Suppression of innate immunity by PRRSV is well-documented which delays a strong and effective VN-antibody response for 4-6 weeks (Lopez et al., 2007; Yoo et al., 2010; Benfield et al., 1992; reviewed in Butler et al., 2014). Specifically in the case of PRRSV, type I IFN expression is suppressed or altered (Buddaert et al., 1998; Calzada et al., 2011; Patel et al., 2010; Wang et al., 2013) but can be reversed using an adenovirus vectors expressing IFN-α (Brockmeier et al., 2009).

One of the first recorded pathological effects of PRRSV infection was thymic atrophy (Rowlands et al., 2003).

This effect is virulence dependent; high path strains cause severe atrophy (Li et al., 2014; Fig. 6). In this situation PRRSV infects the CD14+ APCs as well as CD3+ thymocytes, which causes their apoptosis (Figures 4 and 7). APCs infected in utero could present PRRSV epitopes to developing CD3+ thymocytes (Fig. 7).

Figure 6. Thymic atrophy in piglets infected with high path PRRSV (HuN4).

Studies using Landrace-Yorkshire isolator piglets and fetal piglets infected with the VR 2332 lab strain, provided evidence for a third form of immunopathology. We showed that PRRSV infection resulted in lymphoid adenopathy, hypergammaglobulinemia (Fig. 8A) and rapid differentiation of B cells into Ig-secreting plasma cells (Fig. 8B; Lemke et al., 2004; Sun et al., 2012; Sinkora et al., 2014). This rapid conversion of B cells to plasma cells correlates with the observation that <1% of the elevated IgG levels (Fig. 8A) were virus-specific (Lemke et al., 2004). We later showed that the elevated Ig came from plasma cells expressing an undiversified B cell repertoire (Butler et al., 2007; 2008). This effect can be quantified by measuring the repertoire diversification index (RDI; Fig.9).

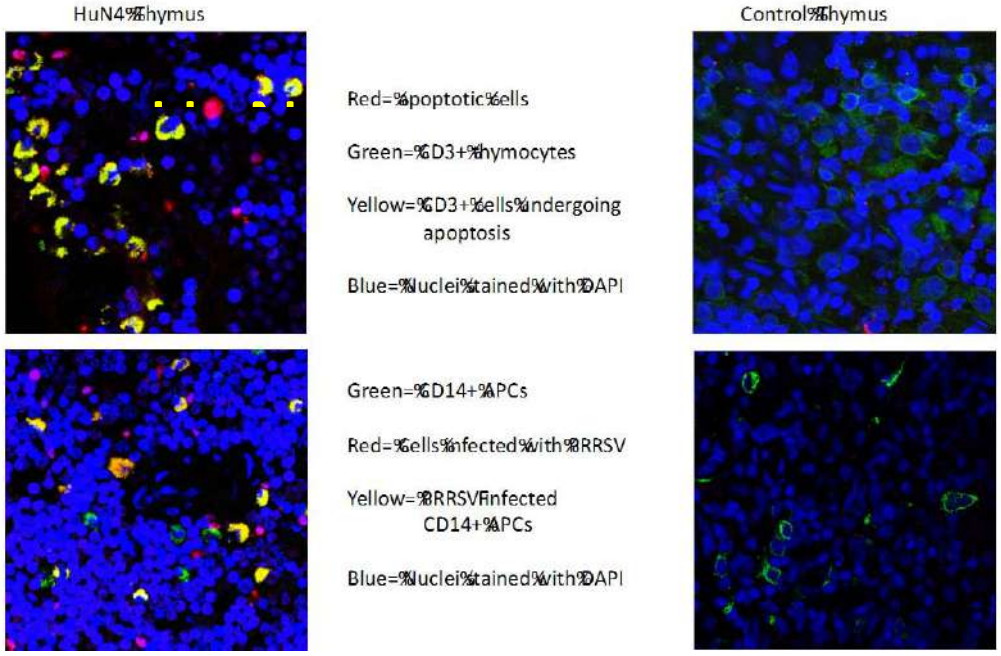

Figure 7. TOP: CD3+ thymocytes infected with PRRSV undergo apoptosis. BOTTOM: CD14+ APCs in piglets infected with HuN4 PRRSV, are infected with PRRSV.

Discussion

We believe that all three of the immune dysregulatory effects described act together to compromise the anti-PRRSV immune response including delaying the production of effective VN-antibodies. First, by interfering with type I IFN production, innate viral immunity is suppressed which delays the recognition by the host that it is infects which delays activation of the adaptive immune system (Fig. 2). Second, by causing apoptosis of PRRSV-infected CD3+ thymocytes (Fig. 4) randomly reduces the size of the pre-immune repertoire so there would be fewer T cells that could

recognize PRRSV, other pathogens and foreign antigens. Evidence in support of this contention is shown in Figure 10 in which infection with high-path PRRSV (HuN4) significantly reduces the proportion of double-positive T cells (CD4+ CD8+) compared to controls. This can also be seen after infection with the low-path CH-1a (Fig. 10B). This results in an alteration of the T cell repertoire, especially Vβ families IV-VI as shown by their restricted spectratype (Fig. 10C). The atrophy shown in Figure 6 could be explained by the loss of infected thymocytes by apoptosis. While this is one possible effect of infection of the thymus, there is yet another. We illustrate in Figure 4 that developing CD3+ thymocytes that encounter antigens during fetal development, are "educated" those eliminating all thymocytes that are presented a self-antigen by an APC during fetal life. Since PRRSV presented by APCs to developing thymocytes would be recognized as self, thereby causing their elimination, maturing T cells leaving the thymus and forming the piglets´ T cell repertoire, would be unable to recognize PRRSV as a pathogen.

The third dysregulatory impact of PRRSV that causes hypergammaglobulinemia and accelerates B cell differentiation to plasma cells (Fig. 8) might allow insufficient time for the somatic generation and selection of high affinity B cells and production of anti-PRRSV antibodies (Fig. 2). This could explain why <1% of the elevated IgG levels was specific for PRRSV (Lemke et al., 2009) and that the plasma cell population responsible is still expressing an undifferentiated B ell repertoire (Butler et al., 2007. 2008; Fig. 9). Furthermore, it is known that high serum levels of IgG are involved in a feedback loop that down-regulates B cell activity (Cerottini et al., 1969; Heyman 2003). We observed this to be the case when maternal IgG provided in colostrum, suppressed Ig synthesis by the suckling piglet (Klobasa et al., 1980). Interestingly, labile factors in colostrum also down-regulate B cell activity (Klobasa et al., 1990; Butler et al., 2019) which may explain why investigators using conventional pigs did not observe the severe hypergammaglobulinemia we observed using isolator piglets (Lemke et al., 2004; Fig.8A).

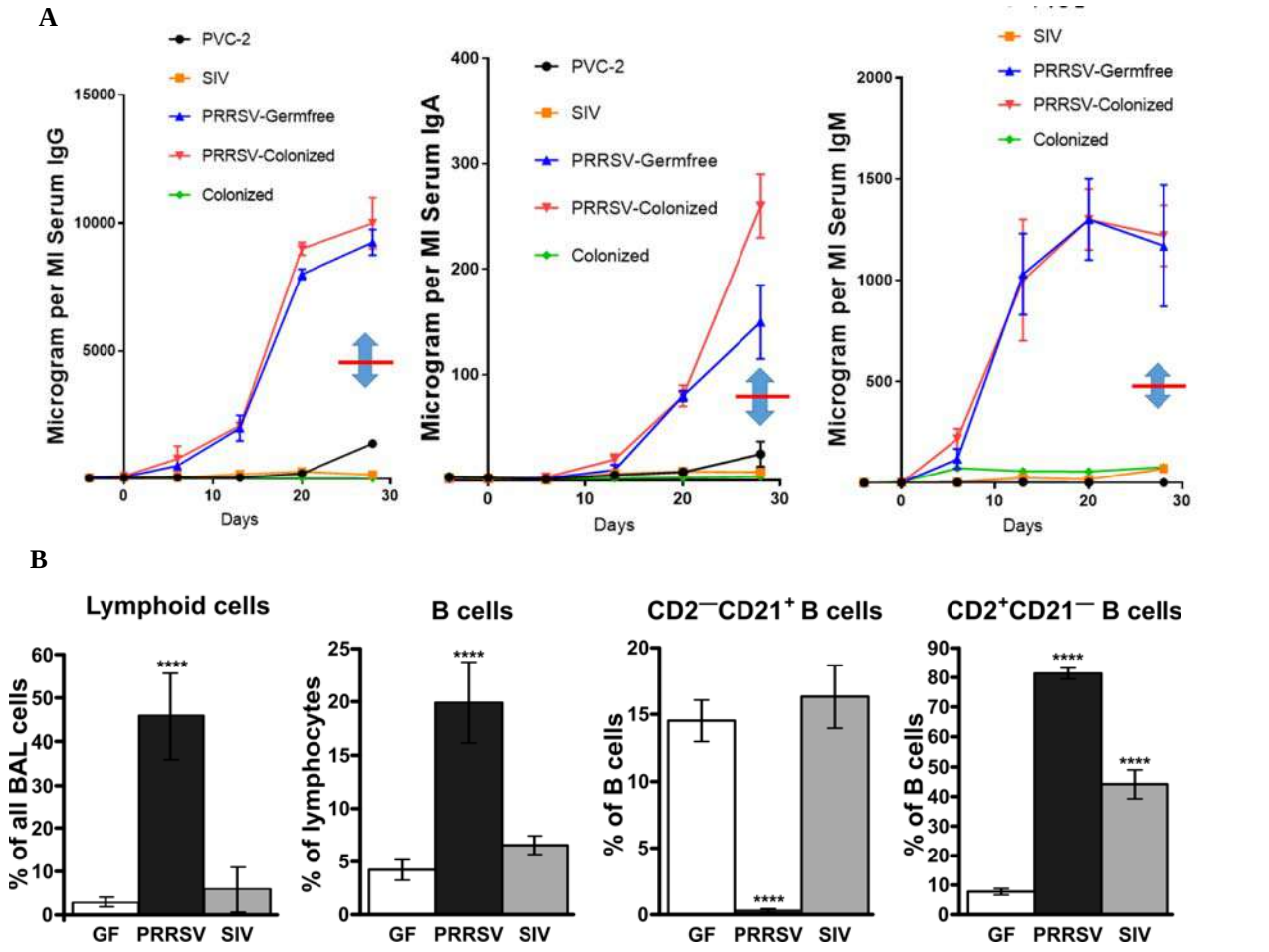

Figure 8. A. Hypergammaglobulinemia in isolator piglets infected with the VR 2332 PRRSV, SIV and PCV-2. Blue arrows with red bars indicate the means Ig levels in age-matched conventionally-reared piglets. B. The expansion of lymphocytes and B cell populations in PRRSV-infected isolator piglets compared to control animals and isolator piglets infected with swine influenza A virus (SIV). CD2- CD21+ is the phenotype for naïve B cells and CD2+ CD21- is the phenotype of plasma cells in swine. From Butler et al., 2019 and Sinkora et al., 2014.

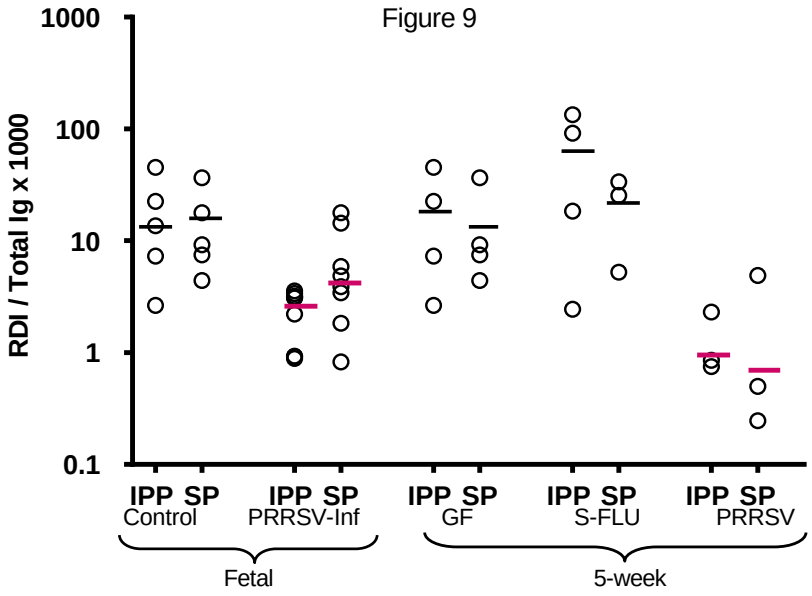

Figure 9. Antibody repertoire development after 35 days in fetal and isolator piglets infected with PRRSV and control piglets. Note that the RDI is a log scale so that the mean RDI for PRRSV-infected fetuses is > 2-fold lower than controls. Similarly, in 35-day old isolator piglets, the RDI is nearly 10-fold lower than in colonized piglets. This is nearly 100-fold lower than colonized piglets infected with swine influenza (S-FLU). SP= spleen and represents the systemic immune system while IPP= ileal Peyer patches, and reflects the mucosal GIT response. In all cases, repertoire development in suppressed in both fetal and isolator piglets infected with VR 2332. From Sun et al., 2012.

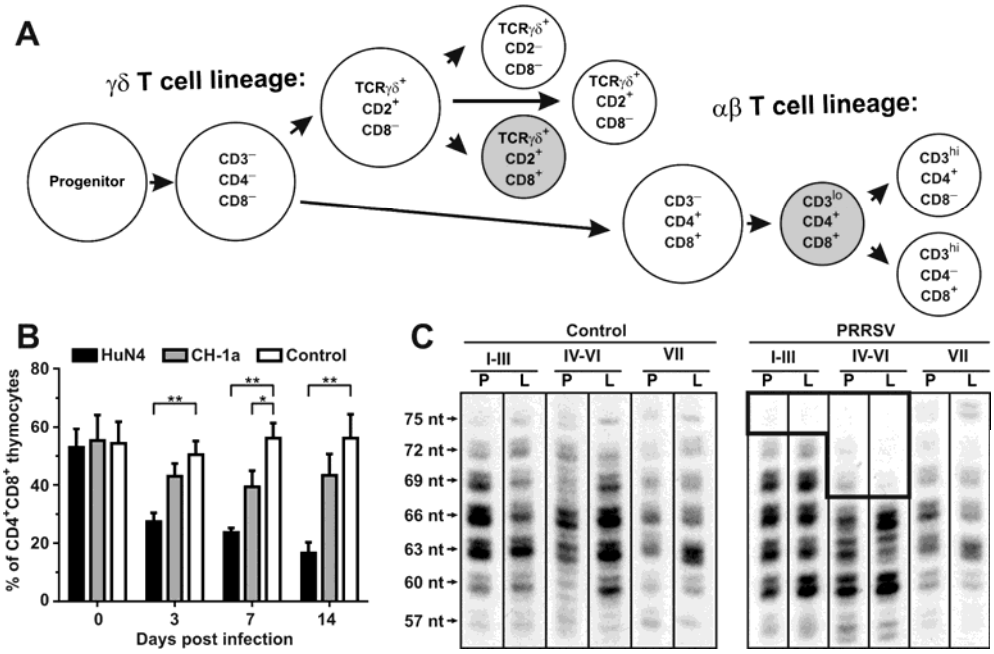

Figure 10. The impact of PRRSV infection on the proportion of double positive (CD4+ CD8+) thymocytes in piglets infected with high-path PRRSV (HuN4), a less virulent form (CH-1a) versus in age-matched control piglets. A. Review of the pathways of thymocyte development. Double positive cells are in gray. B. The proportion of double-positive T cells in control piglets and those infected with high-path PRRSV (HuN4) or low-path (CH-1a) PRRSV. C. Spectratypic analysis of T cells from control and PRRSV-infected piglets. Brackets indicate that certain T cell clones in the Vβ IV-VI families are absent from piglets infected with PRRSV. P= cells from peripheral blood; L= cells from bronchial-alveolar lavage. Roman numerals indicated different V- families of porcine T cells receptors as described by Butler et al., 2005.

So, what are the major take-home messages? First PRRSV is a highly variable RNA virus capable of changing its genome and offering new challenges to the immune system. PRRSV resembles influenza A and FMD but with the added effect of immune dysregulation. A polyvalent influenza A vaccine may soon be available and perhaps such a vaccine might be developed to combat PRRS. In a recent comparative study, live PRRSVs were more effective than killed version (Toman et al., 2019). However, constructing a live polyvalent vaccine moves the issue to uncharted waters. Preventing "abortion storms" might be handled by a separate and complementary approach using hormone therapy to prevent PRRSV-infected sows from aborting their perfectly heathy litters. If placental transfer of PRRSV is responsible for infection of the thymus, which causes thymic atrophy and restricts the T cell repertoire, perhaps understanding how PRRSV is able to cross the placenta and devising a pharmaceutical antidote, might also lead to parallel therapy to control this pandemic viral disease of swine.

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Structure-based antigen design: Targeting transferrin receptors to prevent respiratory and systemic disease of swine

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The Pasteurellaceae family and its importance in pig production

The Pasteurellaceae family is comprised of a group of Gram-negative coccobacilli bacteria that inhabit the respiratory, oral or gastrointestinal tracts of different animal species and can cause a wide range of infectious diseases in animals and humans. The family includes at least three important and well-characterized bacterial genera; namely, *Pasteurella*, *Actinobacillus* and*Glaesserella*that can efficiently colonize the respiratory mucosa of pigs and trigger a chronic respiratory disorder, pneumonia or septicemia in animals of different ages (Figure 1), resulting in important economic losses for the pig industry worldwide.

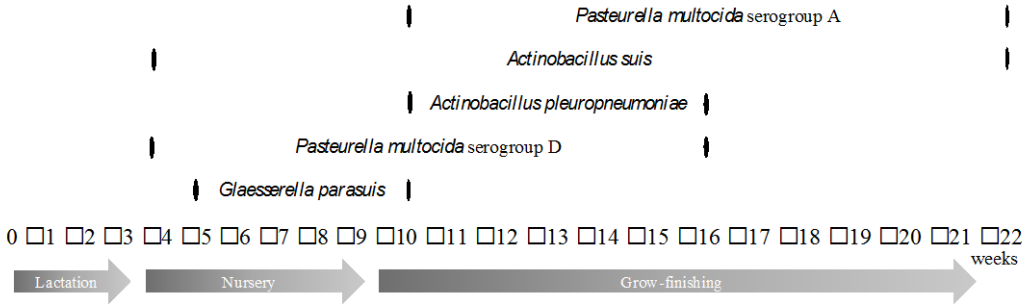

Figure1. Temporal illustration of the periods for observing the diseases in pigs caused by bacteria from the Pasteurellacea family.

Pasteurella multocida serogroup D and A are the two major capsular types that cause respiratory disease in pigs. Toxigenic strains of *P. multocida* (mainly from serogroup D) that secrete dermonecrotic toxin (PMT) are responsible for progressive atrophic rhinitis (PAR)(Foged et al., 1987); a pathological condition of the upper respiratory tract that is initiated in young piglets and significantly reduces the growth performance in the fattening phase (weight gain reduction of 6%) (Donkó et al., 2005). In parallel, toxigenic *Bordetella bronchiseptica*, which is widespread in pig production, cause nonprogressive atrophic rhinitis, a clinical manifestation with minor growth impact in diseased pigs(Jong, 2006).Although some strains of *P. multocida* serogroup A can also express PMT and induce PAR, this capsular type is more commonly associated with lung pathology (bronchopneumonia) (Choi et al., 2003) as a secondary agent of enzootic pneumonia caused by *Mycoplasma hyopneumoniae* infection(Hansen et al., 2010; Pijoan and Fuentes, 1987). However,this trend of pathogenesis appears to be variable, since some clinical strains of *P. multocida* serogroup A are highly virulent and able to produce necrotic and focal bronchopneumonia, diffuse fibrinous pleuritis and pericarditis without any other co-infection(Oliveira Filho et al., 2018).

In the lung milieu, *Actinobacillus pleuropneumoniae* can cause porcine pleuropneumonia, an important inflammatory and highly contagious disease frequently diagnosed in intensive pig farms worldwide. *A. pleuropneumoniae* can be transmitted from the infected sows to their offspring during the second week of life (Vigre et al., 2002), however, the clinical disease during the sucking period or at nursery phase is suppressed by the high levels of maternal antibodies acquired from the colostrum (Krejci et al., 2005) against the homologous serovars transmitted by the sows. As a strategic colonizer of the tonsils, *A. pleuropneumoniae* can occasionally persist undetected in the host, due to its capacity to circumvent the induction of specific antibodies against toxins (Chiers et al., 2002; Sassu et al., 2018), contributing to maintenance of the pathogen in subclinically infected animals with potential for transmission. The most important virulence factors of *A. pleuropneumoniae* are the Apx toxins, which havedifferent degrees of cytotoxicity, haemolytic activity and distribution amongserovars (Frey, 1995; Sarkozi et al., 2015; Schaller et al., 2000). In the lung, the toxins cause tissue damage (APXI, II and III) and erythrocyte hemolysis (APXI and II), resulting in severe edema, inflammation, hemorrhage, necrosis, diffuse fibrinous pleuritis and pericarditis (Ajito et al., 1996; Bertram, 1985; Bosse et al., 2002; Rosendal et al., 1985). Animals that survive infection may have complete

resolutionof lesions, but most frequently they retain focal necrotic areas and/or well encapsulated abscesses with overlying areas of fibrinous connective tissue (Bosse et al., 2002; Frey, 1995; Rosendal et al., 1985). Animals infected with A. *pleuropneumoniae* infection commonly develop pleuritis which leads to substantial losses at the slaughterhouse (Fraile et al., 2010). This lesion is very prevalent (between 25 and 50%) in several countries (Jager et al., 2012). In general, *A. pleuropneumoniae* cause substantial economic losses for the pig industry, mainly due to a decrease in the average daily gain and feed efficiency (Straw et al., 1989), increased mortality during the fattening phase, the cost of medication and veterinary expenses (Hunneman, 1986), as well as carcass condemnation or reduced meat yield in slaughterhouses.

Actinobacillus suis is another member of the genus *Actinobacillus* and an important opportunistic bacterium that can cause disease (systemic inflammatory disorder) in pigs of all ages (Ojha et al., 2010). *A. suis* can adhere to the tonsil's cells through OmpA protein (Ojha et al., 2010) and reside in it as well as on the mucosal surface of the soft palate (Bujold and MacInnes, 2016; Kernaghan et al., 2012). In certain circumstances, which are poorly understood, the bacterium can trigger necrotic glossitis (Sugie et al., 2019), enteritis, abortion, mastitis, metritis, abortion, meningitis, arthritis and sepsis (MacInnes and Desrosiers, 1999). In contrast to *A. pleuropneumoniae*, where a vast knowledge about pathogenesis is available, little is known about the induction disease mechanisms of *A. suis*. Nevertheless, *A. suis*encodes proteins that bind to collagen I and IV, fibronectin and vitronectin (Bujold and MacInnes, 2016)and also secretes two cytotoxic toxins (APXI and APXIII) (Schaller et al., 2000) with similar function to those secreted by *A. pleuropneumoniae*.

In contrast to other members of the *Pasteurellaceae* family, *Glaesserella parasuis*, formerly known as *Haemophilus parasuis* (Dickerman et al., 2020)causes Glasser'sdisease (GD) withoutsecreting toxins. GD is an emergent and worldwide disease that mainly affects pigs in the nursery phase and early fattening stages (Costa-Hurtado et al., 2020). *G. parasuis*has been classified into three groups according its virulence; and strains that are moderatelyor highly virulent can overcome the innate defenses of the host (Cerda-Cuellar and Aragon, 2008; Costa-Hurtado et al., 2013; Frandoloso et al., 2012; Olvera, 2009) and cause a severe systemic inflammatory disease in pigs. Clinically, diseasedpigs develop polyserositis, polyarthritis, meningitis, corneal opacity and optical nerve congestion (Dazzi et al., 2020), which affects the animals performance and causes significant economic losses. The transmission of the bacteria occurs very early (first week) from the infected sows to their offspring (Cerda-Cuellar et al., 2010) and during the lactation period the specific IgGs transferred through the colostrum control the *G. parasuis* infection and avoid the development of GD. In the nursery phase, two events are strongly associated with development of GD: (a) the maternal antibodies decrease to a level that is not able to control bacterial replication, and (b) farms that house animals from multiple sources may have multiple serovarsof*G. parasuis*, making it difficult have all the specific IgGs against capsular antigens required for protection. The bacteria initiate infection by adhesion and invasion of epithelial cells (Bouchet et al., 2009) and can degrade secretory IgAs (Mullins et al., 2011) to evade the specific mucosal immune response from the respiratory tract. Virulent strains are also capable of producing biofilms *in vitro*(Zhang et al., 2014)and forming a biofilm-like structure on the surface of the tracheal epithelium *in vivo* (Bello-Orti et al., 2014) which can contribute to mucosal colonization and antimicrobial resistance (Zhang et al., 2014). Interesting, we have observed recently that *G. parasuis* can efficiently migrate to the maxillary sinus membrane from the respiratory mucosal (*manuscript in preparation*), which is very vascularized and the bacteria can efficiently invade endothelial cells (Frandoloso et al., 2013). Therefore, this pathway can be an alternative door for the bacteria to produce systemic infection, avoiding the contact with the specialized phagocytic cells localized in the lung.

Serology, bacterins, polysaccharide capsules and current vaccines

Established serological schemes for classifying different groups or lineages of bacteria initially simply involved immunizing animals with killed bacteria and using the resulting sera for identifying which group or lineage a particular isolate belongs to. In bacterial species that possess extracellular polysaccharide capsules, such as many members of the Pasteurellaceae, the serological response is predominantly against the polysaccharide capsule, thus is determined by the specific arrangement of biosynthetic genes involve in capsule production. This approach has not only been useful for identifying the overall characteristics of different lineages of bacteria but led to the strategy of developing inactivatedwhole cell vaccines (bacterin), since a robust anti-capsular response is generally very effective at prevention of infection.

The initial efficacy of bacterin vaccines used in the food production industry provided the conceptual basis for developing conjugate-capsular vaccines in humans, where various safety and toxicity issues made simple bacterins an unlikely commercial product. The development of the conjugate capsular vaccine against type B *Haemophilus influenzae* (Hib)(Peter et al., 1991) dramatically reduced the disease, raising the prospects of virtually eliminating the disease by this pathogen (Heath, 1998). The success of the Hib vaccine led to the development of conjugate capsular vaccines against the other key human pathogens causing meningitis, *Neisseria meningitidis* and *Streptococcus pneumoniae.* The development and testing of conjugate capsular vaccines made it very clear that the protection was highly specific, with virtually no cross-protection with other capsular types.

Ongoing epidemiological studies revealed that the conjugate capsular vaccines not only were effective at reducing infection but were also eliminating carriage by the targeted capsular types (Kellner et al., 2008) which resulted in reduced disease in non-immunized individuals. Although this herd immunity phenomenon enhances the efficacy of the vaccines, it also indicates that the selective pressure on the inhabitants of the upper respiratory tract can result in vaccine escape by genetic exchanges in these naturally transformable bacteria, including acquisition of a different capsular type (Croucher et al., 2011). It is also important to consider the potential reservoir of capsular genes from the upper respiratory tract microbiota as illustrated with the more extensive analysis of related commensal species of *Neisseria* that were considered capsule negative but clearly do possess capsule loci that could ultimately lead to additional capsule types in *N. meningitis* (Clemence et al., 2018). Thus, in spite of the demonstrated efficacy of the conjugate capsular vaccines in resulting in dramatic reductions in disease incidence, there is a need for continual monitoring the epidemiology of infections and to continue to explore alternate vaccine approaches.

Although not as extensively studied, the issues raised regarding conjugate capsular vaccines likely apply to bacterins that are prepared from Pasteurellaceae species that cause important infections in food production animals. Since the protection is almost exclusively due to the anti-capsular immune response, the challenge is to select the most appropriate strains for preparation of bacterins and to ensure that the resulting bacterins are, or continue to be, appropriate for the strains that are causing disease on the farm. This challenge is often not met, as the development and use of bacterin vaccines against *G. parasuis* illustrates. There are fifteen serovars that have been classified as high, intermediate or low virulence (Kielstein and Rapp-Gabrielson, 1992) and most bacterins have been prepared against virulent strains that were prevalent in the region originally being targeted. However, a review of clinical disease isolates collected over a 29-year period in Brazil (Espindola et al., 2019) demonstrated that there was a poor match between available vaccines and clinical disease isolates, with nearly 18% due to ‘non-typeable’ strains that represent up to 9 new capsular serotypes. Notably, one also has to be cautious about conclusions regarding the virulence of strains as there can be loss of virulence during growth in the laboratory, that can be restored by passage in animals, especially the native host (Dazzi et al., 2020).

One of the challenges faced when developing vaccines against members of the Pasteurellaceae is the highly efficient natural transformation system in these species(Redfield et al., 2006) that provides a mechanism for vaccine escape by horizontal exchange of target variants from related residents of the upper respiratory tract microbiome. The porcine pathogens *P. multocida, A. pleuropneumoniae* and *G. parasuis* contain all the known genes involved in the natural transformation process and have the required uptake signal sequences (USS) distributed throughout their genomes(Redfield et al., 2006). Published studies have demonstrated natural transformation in *A. pleuropneumoniae*(Bosse et al., 2004)and *G. parasuis*(Li et al., 2016) which also possess an overlapping repertoire of USSs. Antigenic variants of transferrin binding protein B have been shown to be distributed amongst these two species as well as *A. suis*(Curran et al., 2015) but the degree to which recent exchanges contribute to this distribution is uncertain as these proteins have undoubtedly been present in ancestors of the current species. Although we are not aware of published studies demonstrating natural transformation of *P. multocida*, the retention of USS and intact genes required for the process is a strong indication that it has been maintained in this species. In addition to the obvious acquisition of variants in the vaccine target by horizontal exchange, there is also a subtler impact of horizontal exchange that could result in increased virulence of resident strains in the population (Watkins et al., 2015).

Although reduction or prevention of colonization is clearly effective in reducing natural infection and can eventually lead to elimination of the targeted pathogen as illustrated with the use of conjugate capsular vaccines in humans(Kellner et al., 2008), it is not sufficient to prevent disease by toxin-producing bacteria in most models of infection. The direct administration of the bacterial challenge via aerosol or intra-tracheal administration effectively delivers large doses of toxin that can result in substantial tissue pathology before the immune system can effectively deal with the bacteria introduced into the lung. The relative simplicity of direct challenge experiments and the preferred acceptance by regulatory agencies compared to ‘seeder pig’ models (Dee et al., 2018) tends to bias vaccines compositions towards inclusion of toxins, especially in the case of *A. pleuropneumoniae*.

Of the five recognized *P. multocida* serogroups (A, B, D, E and F) (Townsend et al., 2001), vaccines have been developed against porcine strains of*P. multocida* serogroup A and D. The commercial vaccines contain inactivated *P. multocida* strains with or without inactivated toxin formulated with classical adjuvants:aluminum hydroxide (AL)-gel, diethylaminoethyl (DEAE)-dextran and alpha-d-tocopheryl acetate (vitamin E).

Since there are 18 known serotypes of *A. pleuropneumoniae*(Bosse et al., 2018)there is uncertainty regarding the long-term efficacy of three of the major vaccines used globally that target virulent lineages:bacterins with serovar 1, 5 and 7 strains, bacterins with serovar 2, 4 and 5 strains, or bacterin with serovar 1 and 2 strains plus detoxified toxins (APXI, II and III). The fourth major vaccine contains the three inactivated toxins plus an outer membrane protein, for which the loss or replacement by immunological variants may influence the long-term efficacy of the vaccine.

Commercial vaccines are common strategy forcontrolling*G. parasuis* infection. Most globally available vaccines are based on bacterins from one (SV1 or SV5) or two sorovars (SV1 and 6 or 4 and 5) of *G. parasuis*. The recent demonstration that there are more than the 15 capsular type of *G. parasuis* originally identified and that a substantial proportion of disease in Southern Brazil has been due to non-vaccine capsular types(Pires Espíndola et al.,

2019), underscores the limitations of bacterin vaccines. More recently, one attenuated whole cell vaccine based on *G. parasuis* serovar 5 was licensed on North America. No commercial vaccines are available to prevent *A. suis* infection.

Autogenous vaccines are emerging as the most logical short-term solution to control disease outbreaks in farms that are using ineffective commercial vaccines (when the disease-associated strains are not covered by vaccine antigens). On the other hand, the most effective long-term solution is development of more cross-protective protein-based vaccines such as one targeting surface transferrin receptors that is predicted to completely prevent disease by *G. parasuis*(Barasuol et al., 2017; Curran et al., 2015; Guizzo et al., 2018), and could potentially prevent diseases caused by *A. pleuropneumoniae* and *A. suis*as well, since these three pathogens share the same receptor to acquire iron from pig transferrin.

Targeting transferrin binding proteins: an evolutionary and historical perspective

Iron is an essential element for nearly all life forms, likely a consequence of life originating in primordial seas rich in iron nearly 4 billion years ago where it was readily available redox catalyst. In response to the continual decrease in the levels of iron in the seas and oceans over time, systems for the capture and transport of iron into the cell became essential for survival. It is likely that simple organic acids were initially sufficient for capture and transport of iron into the cell. However, as the competition grew for the decreasing levels of available iron, more complex molecules with higher binding affinities for the ferric form of iron appeared. The complex iron binding molecules, termed siderophores (Neilands, 1981), were synthesized and secreted from the cell and, after binding iron, the resulting iron-siderophore complex was captured by surface receptors and transported into cell (Figure 2). The diverse molecular structures of siderophores dictate very specific binding of the iron-siderophore complex by cognate surface receptors.

Evidence for early life on earth primarily comes from stromatolites, structured microbial communities that include cyanobacteria (Reid et al., 2000), highlighting the fact that the capacity of prokaryotes to form microbial communities developed early on. Microbial communities on modern day sea sediment were shown to consist of relatively few siderophore-producing bacteria with many ‘cheaters’ or ‘pirates’ (Figure 2) that could use the resulting iron-siderophore complexes (D'Onofrio et al., 2010).

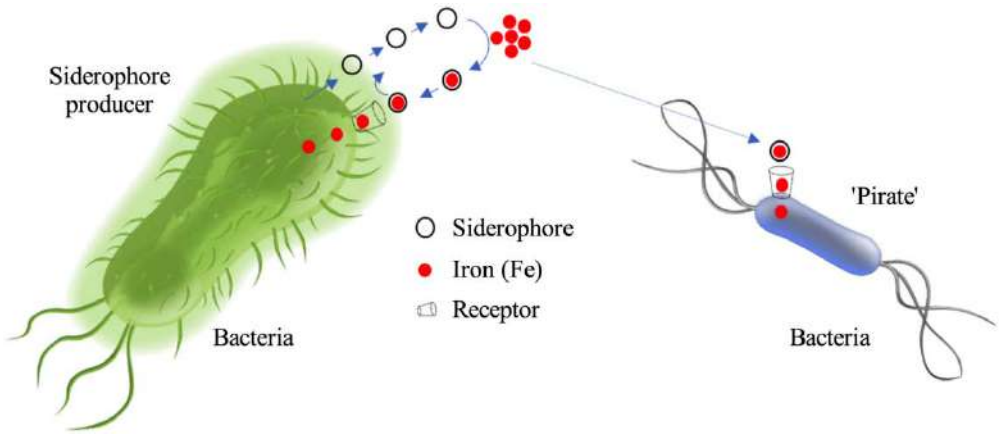

Figure 2. Iron acquisition in microbial communities. Bacteria possessing uptake systems for specific iron-siderophores can utilize (pirate) siderophores produced by other bacteria.

Although there is considerable discussion and debate on the factors responsible for establishing and maintaining this type of relationship between siderophore producer and cheater in different ecological settings (Kramer et al., 2019), it clearly is not uncommon in microbial communities including those present on mucosal surfaces of vertebrate hosts.

The identification of bacterial surface receptors that directly bind and utilize the host glycoproteins transferrin and lactoferrin as a source of iron for growth (Gonzalez et al., 1990; Ogunnariwo and Schryvers, 1990; Schryvers and Morris, 1988a, b), indicated that there was a more efficient mechanism for non-siderophore producing bacteria to acquire iron for growth on the mucosal surfaces of the upper respiratory and genitourinary tracts. The demonstration that these receptors were essential for survival on the mucosal surface and that transferrin was present at higher levels than lactoferrin (Anderson et al., 2003; Cornelissen et al., 1998), underscore the fact that these receptors evolved for survival on the mucosal surfaces where these bacteria exclusively reside. The observation that the transferrin receptors were exquisitely specific for host transferrin (Gray-Owen and Schryvers, 1993; Schryvers and Gonzalez, 1990) has been shown to be due to selective forces on the evolution of transferrin by the receptor proteins which over a 40 million year period has defined the receptor specificity amongst primates (Barber and Elde, 2014). This also suggests that the presence of receptor homologues in pathogens of poultry (Ogunnariwo and Schryvers, 1992) indicates that these receptors have been present for over 320 million years when the ancestors of birds and mammals diverged.

An evolutionary perspective is useful to help understand several features that make transferrin receptors ideal vaccine targets.Since the bacterial species in the Pasteurellaceae, Neisseriaceae and Moraxellaceae families that possess surface transferrin receptors have been adapting to their host over a long period of time they have become dependent upon these receptors for survival (no longer possess a repertoire of siderophore receptors). The combination of efficient mechanisms of genetic exchange through natural transformation (Mell and Redfield, 2014; Redfield et al., 2006) and a pre-existing global diversity of the surface receptor proteins (Curran et al., 2015) is sufficient to counter the immune responses that may impact survival on the mucosal surface of the host. In other words, these bacteria may primarily rely on 'shuffling the deck' rather than mechanisms for rapidly generating escape variants. These principles, along with the realization that the receptor proteins will always be expressed in the normal iron-limited environment of the host, provide the foundation for the concept that with a limited number of our engineered vaccine antigens we could potentially eliminate *A.pleuropneumoniae, A. suis* and *G. parasuis* from their porcine host.

Although the transferrin receptors clearly arose to provide an advantage for survival in the upper respiratory and/or genitourinary tract of their vertebrate host, it also provides the bacteria access to a continual supply of iron if the mucosal epithelial barrier is breached. This explains why the bacteria from the Pasteurellaceae, Neisseriaceae and Moraxellaceae families can be primarily commensal bacteria under most circumstances yet be important pathogens of humans and food production animals (Morgenthau et al., 2013). It also underlies the rationale for targeting the transferrin receptor for prevention of invasive infection which prompted earlier efforts at development of a vaccine for prevention of meningitis and sepsis by*N. meningitidis*(Lissolo et al., 1995) that were abandoned after a Phase 1 trial in humans. Recent developments and renewed efforts at development of vaccines derived from the transferrin receptors illustrate that this represents a missed opportunity, as the unique attributes of these receptors make them ideal targets for vaccine development.

Structure designed vaccines for targeting transferrin receptors

The transferrin receptors in the Pasteurellaceae, Neisseriaceae and Moraxellaceae families clearly arose as a modification of the siderophore receptors in Gram-negative bacteria that preceded the transferrin receptors. In the Gram-negative bacteria the iron-siderophore complex is bound by a specific surface receptor embedded in the outer membrane, and the iron-siderophore complex has to be transported across two membranes to enter the cell, a process shown to be dependent upon the*tonB* gene (Bagg and Neilands, 1987; Kadner and McElhaney, 1978; Postle, 1990) (Figure 3, left section). Energy from ATP hydrolysis is transduced to the outer membrane receptor by the TonB complex to drive the transport of the iron-siderophore across the outer membrane, which is then shuttled to an inner membrane transport complex by a specific periplasmic binding protein. Transport across the inner membrane into the cell is mediated by an inner membrane transport complex.

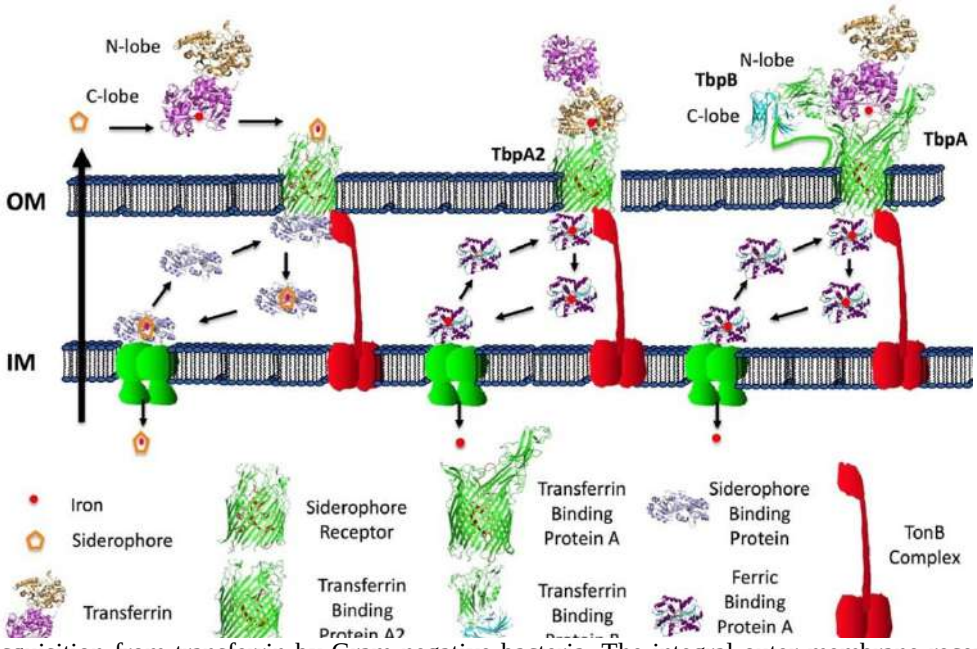

Figure 3. Iron acquisition from transferrin by Gram-negative bacteria. The integral outer membrane receptor proteins for transporting the iron siderophore complex (left),or extracting iron from transferrin and transporting it across the outer membrane (middle and right), use energy derived from the TonB complex to drive transport. The iron-siderophore complex (left) or ferric ion (middle and right) are subsequently bound by a periplasmic binding protein and shuttled to an inner membrane ABC transport complex that uses ATP hydrolysis to transport the iron-siderophore or ferric ion into the cytoplasm.

It is likely that the ancestral transferrin receptor was comprised of a single TonB-dependent receptor protein similar to the TbpA2 receptor (Figure 3, middle section) present in some strains of *P. multocida*(Ogunnariwo et al., 1991) and *Histophilus somni*(Ekins and Niven, 2002), although TbpA2 binds to the C-lobe instead of the N-lobe and is likely a more recent development as it has only been identified in bovine pathogens. The prototypical transferrin receptor (Figure 3, right section) has a surface lipoprotein component (TbpB), with a long (> 40 amino acids) anchor peptide region that enables it to extend far from the outer membrane surface to capture iron loaded transferrin and then transfer it to TbpA, for removal and transport of iron across the outer membrane.

The TbpB protein is an attractive target for vaccine development due to its accessibility to antibodies at the cell surface and the ability to readily produce substantial quantities of soluble and functional recombinant protein in the cytoplasm of *E. coli*, suitable for commercial production. Once the structures of TbpB became available (Calmettes et al., 2012; Moraes et al., 2009), it became possible to design mutant TbpBs defective in binding transferrin that could potentially be superior antigens by preventing masking of important epitopes by transferrin during immunization. A mutant TbpB with substantially reduced binding of porcine transferrin due to a conservative change in the side chain of a single amino acid, Y167A, provided complete protection against infection by *G. parasuis*(Frandoloso et al., 2015) whereas the native TbpB and a commercial vaccine product provided vastly inferior protection from infection.

Notably, an analysis of the global diversity of the TbpB present in *G. parasuis*, *A. pleuropneumoniae* and *A. suis* indicated that the diversity was not strongly associated with species, geographical region or time of isolation (Curran et al., 2015) suggesting that the diversity is old (not primarily due to recent mutation) and that there has been extensive exchange between the three targeted species. In addition, there were three main clusters of diversity primarily associated with the transferrin binding N-lobe of the protein, with relatively little diversity within each cluster, but substantial variation between the clusters. These results strongly suggested that a maximum of three mutant TbpBs would be able to induce a protective immune response that would be effective against all strains of the three targeted pathogens (Figure 4). A subsequent study demonstrated an effective cross-protective response within one of the phylogenetic clusters(Guizzo et al., 2018), and that not all mutant TbpBs will provide superior protection (Figure 4). These results do not suggest that it will not be possible to develop a broadly cross-protective vaccine, but that it will require additional effort and testing to determine the composition of engineered proteins that can induce the broadly cross-protective immune response.

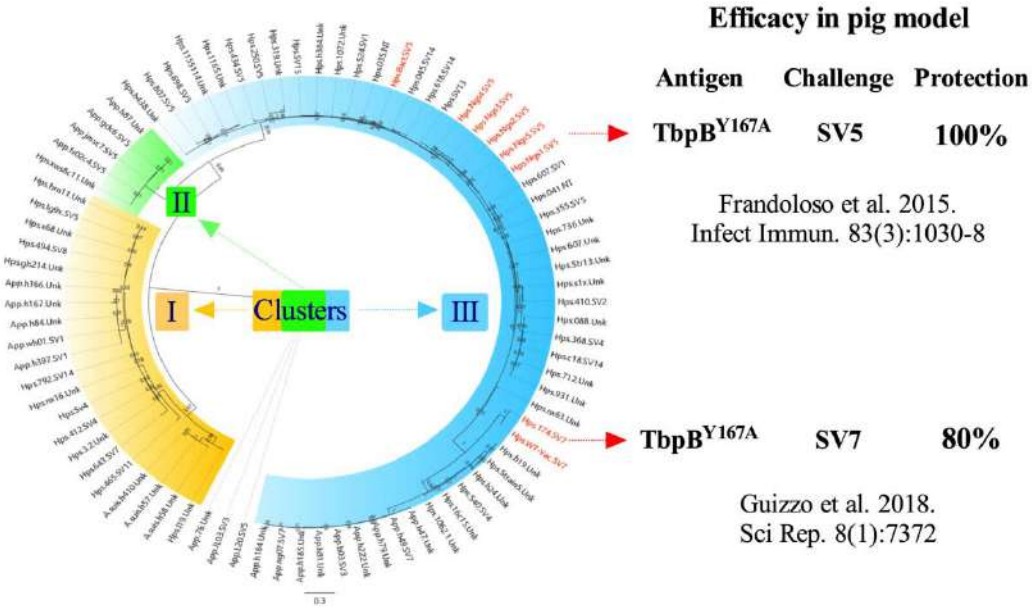

Figure 4. Sequence diversity of TbpBs from porcine pathogens. Maximum likelihood tree demonstrating the overall diversity of TbpBs from *G. parasuis*, *A. pleuropneumoniae* and *A. suis*. Leaf labels identify the strains from which TbpB sequences were obtained and indicate their species and serovar, if known (NT = Nontypeable, Unk = Unknown). The sequences are rooted by the three sequences with a white background, which are the secondary TbpB-like genes. The sequences clustered into three main groups (Group I = yellow background, Group II = green background, Group III = blue background) with high confidence. The branch support values are displayed. The efficacy of vaccines based on TbpB^{Y167A} which belongs to cluster III is highlighted in the right painel. Figure adapted from Guizzo et al. (2018).

Although protection from infection is the primary goal of most vaccine development programs and is the feature that most regulatory agencies are looking for, the ability to prevent or eliminate colonization is a much more effective preventative measure. Since colonization precedes natural infection, a vaccine that prevents colonization will

also eliminate disease, even though it may not be effective in direct challenge models where artificially high levels of bacteria are administered to a target organ (i.e. aerosol or intra-tracheal injection of bacteria into the lungs). The fact that the transferrin receptors are required for survival on the mucosal surface, thus would virtually always be expressed unless there was a sudden supply of iron (i.e. nose bleed), makes them an ideal target for prevention of colonization. Recent studies by our group has demonstrated that immunization with a vaccine preparation containing the $TbpB^{Y167A}$ protein not only prevented infection by *G. parasuis* but also eliminated the natural colonization by this bacterium in the pig barn (Frandoloso et al, manuscript in preparation).

In order to understand how our vaccine based on the $TbpB^{Y167A}$ protein could protect pigs challenged with lethal doses of *G. parasuis* SV5 and SV7, we conducted several *in vitro* studies(Barasuol et al., 2017; Frandoloso et al., 2015; Guizzo et al., 2018). As illustrated in Figure 5, the protective mechanism provided by the $TbpB^{Y167A}$-based vaccine is entirely humoral. Briefly, immunized pigs (two-dose vaccine protocol) produce high titers of systemic IgGs capable of: (a) blocking the interaction between *G. parasuis* TbpB and porcine transferrin which restricts the iron uptake, and consequently, *G. parasuis* depletes its iron reserves and cannot replicate(Figure 5A); (b) efficiently activating the classical pathway of the complement system (Figure 5B), a powerful weapon to killing *G. parasuis*; and (c) efficiently opsonizing *G. parasuis*, increasing the efficiency and speed of phagocytosis (Figure 5C). Altogether, these results provide strong evidence that a mutant TbpB vaccine preparation could be developed to eliminate the three targeted pathogens from commercial pig barns, even ones with less effective biosecurity features.

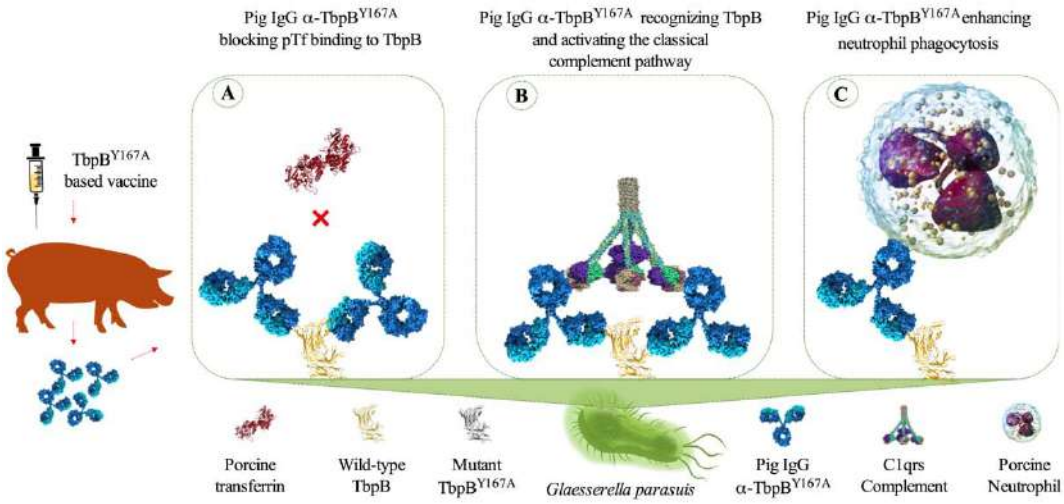

Figure 5. Illustration of the Immunobiological mechanisms mediated by anti-TbpB IgGs. A) Neutralization of the iron uptake receptor by specific IgG anti-TbpB. B) Activation of the classical of the complement system pathway. C) Opsonophagocytosis. All these mechanisms have been demonstrated in our previous studies (Barasuol et al., 2017; Guizzo et al., 2018).

The TbpA protein (Figure 3, right segment) is also a logical target for vaccine development due to its essential role in the iron acquisition process and limited variation in sequence relative to TbpB. However, integral outer membrane proteins are not suitable for commercial production of a protein-based vaccine due to the need for lipids or detergents to maintain solubility and native conformation in aqueous solution. To overcome this limitation, we have developed an approach for displaying the surface loops of TbpB on a scaffold derived from the TbpB protein (Fegan et al., 2019) which may ultimately yield hybrid antigens that provide cross-protection against both of the proteins that comprise the transferrin receptor.

A TbpB based vaccine – one vaccine to conquer all

The experimental evidence to date indicates that there are three phylogenetic clusters of TbpB diversity among the three porcine pathogens *G. parasuis*, *A. pleuropneumoniae* and *A. suis*(Curran et al., 2015), and that a single engineered TbpB is sufficient to induce a fully cross-protective response within a cluster (Guizzo et al., 2018), suggesting that vaccine comprised of 3 mutant TbpBs should providecomprehensive cross-protection against all known TbpB variants (Figure 4). Since the mutant TbpBs not only provide protection from infection, but are capable of eliminating natural colonization, it should be possible to implement a properly designed vaccination program to eliminate these three pathogens from commercial pig production facilities. To date, all new sequences of TbpB variants from these three species fall within the three known phylogenetic clusters, suggesting that it is unlikely that vaccine escape can be accomplished by existing TbpB variants within these three species. However, it may be prudent to search

the upper respiratory microbiome of various breeds of pigs for other bacterial species that might possess transferrin receptors with diverse TbpB variants that could serve as a potential reservoir for vaccine escape.

Although the TbpB protein is not essential for iron acquisition from transferrin under laboratory conditions, the TbpB protein has been shown to be essential for survival and disease causation in pigs (Baltes et al., 2002), suggesting that TbpA will not be able to compensate for loss of functional TbpB for evasion of the vaccine-induced immune response. Even if that were possible, designing hybrid antigen vaccines targeting TbpA (Fegan et al., 2019) could overcome this possibility, as recent results suggest that comprehensive protection can be achieved by optimizing this approach (Qamsari et al., 2020).

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Gut Health and the Post-Weaning Transition in Piglets

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Introduction

A gastrointestinal tract (GIT) that functions in an optimum way clearly is of importance to the overall metabolism, physiology, disease status and performance of pigs of all stages of growth and development, and especially in the sensitive post-weaning production period. Disruptions (dysbiosis) in the GIT after weaning caused by internal and external influences can cause large economic losses in the pork industry, therefore the period after weaning generates much interest. Diseases and conditions of the GIT that can cause losses, for example, post-weaning diarrhoea, have to some extent been controlled traditionally by the use of antimicrobial compounds administered in the feed and (or) water, such as antibiotics and supraphysiological levels of trace elements such as zinc and copper. However, legislation and judgements in various parts of the world together with general efforts to reduce the use of these compounds have caused a reassessment of measures to influence GIT structure and function (i.e., 'gut health'), and have caused unparalleled interest in alternative strategies (e.g., genetic, dietary, management, environmental, veterinary) to effectively manage the GIT under conditions of external and internal challenge. It is important that the pork industry continues to explore and understand the numerous factors that influence GIT after weaning.

What is gut health?

'Gut health' was defined originally in human medicine (Bischoff, 2011) and is now ubiquitous with respect to pig health and production, especially in the post-weaning period (e.g., Lallès et al., 2004; 2007; Lallès, 2008; de Lange et al., 2010; Pluske, 2013; Heo et al., 2013; Lindberg, 2014; Kogut & Arsenault, 2016; Celi et al., 2017, 2019; Jayaraman & Nyachoti, 2017; Moeser et al., 2017; Pluske et al., 2018; Pluske & Zentek, 2019). 'Gut health' is also regularly used in the popular press and in on-line articles and reports and is a common point of discussion and debate at meetings, forums and workshops. The term is therefore used in many different contexts and applications. Nevertheless, Bischoff (2011) remarked that 'gut health' can be defined as 'a state of physical and mental well-being in the absence of GI (gastrointestinal) complaints that require the consultation of a doctor, in the absence of indications of or risks for bowel disease and in the absence of confirmed bowel disease'. Bischoff (2011) said that the prevention or avoidance of GIT disease forms an integral part of our understanding of 'gut health', and defined five major criteria that could form the basis of a definition of 'gut health': (1) effective digestion and absorption of food, (2) absence of GI tract illness, (3) normal and stable intestinal microbiome, (4) effective immune status, and (5) status of well-being. In this regard, Pluske and Zentek (2019) commented that there is sometimes a tendency to associate 'gut health' with bacterial and (or) viral pathogens that cause, either clinically or sub clinically, illness to pigs after weaning, and indeed at any stage of the production cycle. However, 'gut health' can be compromised in the absence of any diseases in the GIT and expression of clinical disease. Low feed intake after weaning, for example, caused by maternal separation and other psychosocial stressors including transportation, mixing, fighting and the establishment of a new social hierarchy, and (or) immunological stressors such as vaccination, can cause inflammation and dysbiosis in the GIT (Pluske et al., 1997; McCracken et al., 1999; Spreeuwenberg et al., 2001). In turn, GIT barrier function is compromised (Wijtten et al., 2011; Kim et al., 2012; Moeser et al., 2017; Modina et al., 2019) and the microbiome and its functions can change (Guevarra et al., 2018), negatively impacting on the overall 'gut health' of the young pig.

Separate to these stressors impacting on 'gut health' after weaning, the (usually abrupt) change from sows' milk to a dry, solid feed offered to pigs that occurs adds additional challenges in the post-weaning period, concerning particularly the digestive and absorptive processes and impacts on the microbiota (microbiome) and immune system, especially the innate immune system (Pluske et al., 2018). At a deeper level, and as summarised by Xiong et al. (2019), weaning significantly down-regulated the expression of proteins involved in the tricarboxylic acid cycle, β-oxidation, and the glycolysis pathway in the upper villus and middle villus of the jejunum in early-weaned pigs, but up-regulated proteins involved in glycolysis in crypt cells. In the post-weaning period, the expression of proteins related to various cellular metabolic or biological processes, such as energy metabolism, protein amino acid glycosylation, ion transport, mTOR signalling pathway, and differentiation and apoptosis, were reduced in jejunal-differentiated epithelial cells (villus upper cells). Proteins involved in the respiratory electron transport chain, Golgi vesicle transport, protein glycosylation, as well as the metabolism of nutrients such as lipids, monosaccharides, and nucleotides, were also down-regulated in the jejunal-differentiating epithelial cells of piglets during this period. These results indicate that weaning influenced energy metabolism, cellular macromolecule organisation and localisation, and protein metabolism, thereby further impacting the proliferation of intestinal epithelial cells after weaning. In addition, polyamine metabolism and ornithine decarboxylase expression were also altered by weaning.

Collectively, an understanding of the composition of the microbial community in the GIT before and after weaning and its functional capacity during the post-weaning transition is important for pig production and veterinary and nutritional practices.

The microbiome and gut health after weaning

The resident GIT microbiota provides the pig with many functions including improved energy harvesting capacity, the production of short-chain fatty acids, production of vitamin K, polysaccharide fermentation, and enhanced resistance against pathogenic bacteria. The pig GIT contains a diverse and complex microbial community, with the total number of bacteria in the pig colon been estimated as 10^{10} – 1 x 10^{11} per gram of content; more anteriorly, populations are at a lower density (Gaskins, 2001). As mentioned previously, weaning causes profound physiological changes in the structure and function of the GIT corresponding to the GIT microbiota undergoing a very quick ecological succession upon induction of these various factors during the weaning (transitional) period. This *microbial shift* (Kim & Isaacson, 2015) is influenced strongly during weaning by the sudden change in diet from simple to more complex nutrient sources, which effects digestion and absorption capacity of the small intestine and hence influences growth and feed efficiency.

The weaning period exposes young pigs to thousands of new bacterial species, which will play an important role in establishing an adult-like microbiota later in life (Kim et al., 2011; Isaacson & Kim, 2015). Early-life microbial exposure is of particular importance to growth, development of immune system and health (Dou et al., 2017), and may be able to be used to predict a disease outcome ('gut health'). In this particular experiment, the GIT bacterial community was assessed for diversity and composition during the suckling period and then associated with differences in susceptibility of pigs to post-weaning diarrhoea. Using a molecular characterisation of faecal microbiota with CE-SSCP fingerprint, Next Generation Sequencing and qPCR, diarrhoeic and healthy pigs could mainly be discriminated as early as postnatal day (PND) 7, i.e. 4 weeks before the post-weaning diarrhoea actually occurred. At PND 7, healthy pigs (i.e., healthy after weaning, showing no diarrhoea) displayed a lower evenness and a higher abundance of *Prevotellaceae*, *Lachnospiraceae*, *Ruminocacaceae* and *Lactobacillaceae* compared to the diarrhoeic pigs. Regression analyses indicated that these bacterial families were strongly correlated to a higher *Bacteroidetes* abundance observed in PND 30 healthy pigs one week before the diarrhoea. These results emphasise the potential of early microbiota diversity and composition as being an indicator of susceptibility to post-weaning diarrhoea (Dou et al., 2017), and provides potentially tangible, practical ways to positively impact 'gut health' after weaning.

Additionally, the establishment of a beneficial microbiota is important during the weaning stage because piglets still have an immature immune system and depend on sow's milk to prevent colonisation and overgrowth of opportunistic pathogens (Castillo et al., 2007). Therefore, understanding GIT microbial succession during the weaning transition, and how different factors such as diet, stress and housing influence gut microbial shifts in association with enhanced 'gut health', growth performance and well-being of pigs, is critical. In turn, such information may be able to be used to assist with decision-making regarding the use of different diet compositions, antimicrobial compounds, and (or) selected feed additives.

In this regard, Guevarra et al. (2018) performed 16S rRNA gene and whole metagenome shotgun sequencing of DNA from faecal samples from healthy piglets during weaning to measure microbiome shifts, and to identify the potential contribution of the early-life microbiota in shaping piglet health with a focus on microbial stress responses, carbohydrate and amino acid metabolism. Major findings were that the faecal microbiome of sucking piglets showed higher relative abundance of bacteria in the genus *Bacteroides* with abundant gene families related, not surprisingly, to the utilisation of lactose and galactose. *Prevotella* and *Lactobacillus* were enriched in weaned piglets (Figure 1) with an enrichment for the gene families associated, again unsurprisingly, with carbohydrate and amino acid metabolism. In addition, functional capacity of the faecal microbiome showed higher abundances of genes associated with heat shock and oxidative stress in the metagenome of weaned piglets compared to nursing piglets.

Inflammation of the GIT can be a negative outcome from the post-weaning milieu, and although the impacts of localised GIT inflammation are well recognised (summarised in Moeser et al., 2017; Pluske et al., 2018), less is known about its impacts on the GIT microbiome after weaning. In a recent important study, the mechanisms by which GIT inflammation contributes to imbalance of the microbiota has been proposed (Zeng et al., 2017). Under intestinal inflammatory conditions, the host responds by producing reactive oxygen species such as nitric oxide (NO) that is rapidly converted to nitrate (NO_3^-) when released in the lumen. In turn, the nitrate-rich environment is conducive for the growth of *Enterobacteriaceae* that encodes for nitrate reductase genes (Winter et al., 2013). Some pathogens within *Enterobacteriaceae*, namely *Salmonella enterica* serovar Typhimurium and enterotoxigenic *E. coli* (ETEC), induce intestinal inflammation in pigs which disrupts composition (Héctor et al., 2013). For example, in a piglet model of *Salmonella* Typhimurium infection, Arguello et al. (2019) concluded that the host response to infection (immune response and metabolic changes) could be a major contributor to the depletion of commensal/beneficial inhabitants of the intestinal tract (*Lactobacillus*, *Bifidobacterium*, *Prevotella* or *Megasphaera*), and the increase of synergists of infection such as *Akkermansia* or *Citrobacter*. The relative abundance of these synergists in the ileal microbial ecosystem of the post-weaned pig was found to be is positively correlated to the degree of epithelial damage in the

ileum. Therefore, inflammation of the GIT caused immediately by perturbations linked to weaning begins a cascade of events including adverse alterations to the GIT microbiome, which appears to favour the growth of pathogenic bacteria, especially members of *Enterobacteriaceae* (Guevarra et al., 2019).

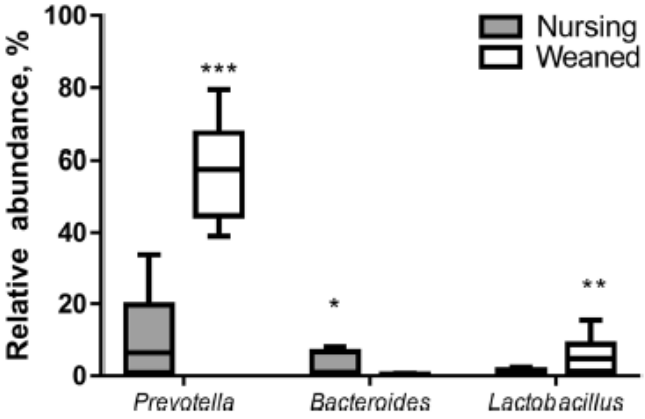

Figure 1. The relative abundance of the significantly different taxa between Nursing and Weaned piglets at the genus level. The interquartile range is indicated by the outer bounds of the boxes, and the median is indicated by the black midline. The whiskers represent the minimum and maximum values. The [P < 0.001], [P < 0.01] and [P < 0.05] were indicated as [***], [**] and [*], respectively (from Guevarra et al., 2018).

Diet, feed additives and gut health after weaning

The development of management and feeding strategies to optimise GIT development and health in newly-weaned pigs in order to improve growth performance and reduce morbidity and mortality, while either reducing reliance on antimicrobial compounds or not having their availability at all, is essential for the sustainability of the pork industry. It is therefore necessary to find combinations of feed ingredients, either alone or in combination with feed additives acceptable for use, that are effective in amending the post-weaning growth check and reducing the incidence and severity of digestive problems frequently encountered (Pluske, 2013). Given the considerable advances already made in the understanding of intestinal nutrient utilisation and metabolism, de Lange et al. (2010) stated at the time that, "a complimentary goal in post-weaning nutrition should be to formulate young pig diets with the specific task of optimising the growth, function and health of the GIT". Since then, the scrutiny associated with using antimicrobial compounds has become even more pronounced, meaning there is even more interest in the use of feed-related strategies to assist with optimisation of 'gut health' in the post-weaning period.

There is a plethora of reviews, papers and articles describing the effects of post-weaning nutritional interventions and changes on 'gut health'. Given that the GIT of the young weaned pig is undergoing rapid changes in size, protein turnover rates, microbiota mass and composition, and quick and marked alterations in digestive, absorptive, barrier and immune functions, then it is problematic to suggest that a single dietary-related strategy can be effective in optimising 'gut health' health in different groups of pigs that are managed under wide ranging conditions of housing, management, feeding and health status. Therefore, a direct 'like-for-like' replacement of antibiotic growth promoters, prophylactic antibiotics and (or) pharmacological levels of zinc oxide (ZnO) with for example, a single feed additive, is unlikely. This emphasises the need to explore underlying mechanisms when evaluating the functional properties of feed ingredients and feed additives, so that a better understand can occur to achieve the optimal response to dietary interventions (Pluske, 2013). Therefore, and currently, it is unlikely that there is any single substance that could reliably and repeatedly replace the function of in-feed or in-water antibiotics. Since the growth benefit found from feeding antibiotics is achieved through many different effects on the GIT, the strategy for replacing them will depend on a combination of nutritional, management, housing, health and (or) husbandry factors. There is also considerable inconsistency in the experimental and (or) commercial outcomes of the many alternatives evaluated, which makes it difficult to judge the efficacy or otherwise of a particular additive (Pluske, 2013).

Amongst the high quality and novel feed ingredients (e.g., insoluble fibre, plasma protein) effective feeding strategies (e.g., lower protein diets) and feed additives that are available, the organic acids have been widely used over time as feed additives for their positive effects on growth efficiency. They are generally considered a valid tool for use in the post-weaning period, more so than in growing-finishing pigs, although expectedly there is a relatively large variation in responses due to various factors such as type and dose of organic acids used, supplementation duration, type of diet and buffering capacity, hygiene and welfare standards, health status, and age of the animals. In a post-weaning

meta-analysis study, Tung & Pettigrew (2006) reported that the improvements of growth rates were 12.2% and 6% for the first 2 weeks or 4 weeks post-weaning respectively, while the enhancement was lower for growing (3.5%) or finishing (2.7%). A full and recent review of different organic acids can be found in Tugnoli et al. (2020).

Of course, there are many other feed additives available for use to modify different aspects of 'gut health', and these are covered in many reviews. Liu et al. (2018), in their extensive review, commented that there are a number of feed additives that potentially may be used in diets fed to pigs, but the main challenge with all of these additives is the fact that results obtained so far have been inconsistent, and especially in field conditions. The reason for this inconsistency may be that efficiencies of each additive are diet dependent and also dependent on the health status of the animals (e.g., Li et al., 2018). Nevertheless, a meta-analysis conducted by Vanrolleghem et al. (2019) evaluated the use of potential dietary feed additives (pDFA) with antibacterial effects and their impact on the performance of weaned piglets, versus a positive control (a diet containing a therapeutic antibiotic or antibiotics). Twenty-three peer-reviewed *in vivo* studies, comprising 50 trials, were identified between January 2010 and January 2017. Five classes of pDFA were formed: antimicrobial peptides, chitosan, lysozyme, medium-chain fatty acids/ triglycerides, and plant extracts. Mixed-effect meta-analyses with type of pDFA as the fixed effect were performed for average daily gain and feed conversion ratio. The results of the meta-analysis showed that adding a pDFA at weaning can improve these performance indicators compared to a Negative control, with no overall significant difference to the Positive control. However, this is only a small evaluation and excludes numerous other feed additives that purport to improve 'gut health' such as probiotics and prebiotics (Liao and Nyachoti, 2017).

Feed processing and gut health

Finally, feed processing and diet manufacturing also play important roles in 'gut health'. The benefits of feed processing in terms of animal performance and economics are apparent; however, feed processing optimisation in the future will also likely be impacted by the same issues as mentioned previously about antimicrobials. For example, feed processing should increasingly consider dietary approaches (ingredients and physical characteristics) for maintaining a healthy and functional GIT. The need to achieve high physical quality and to reduce potential levels of feed-borne pathogens such as *Salmonella* has led to the application of relatively high conditioning temperatures during conventional pelleting processes, but this is a practice that does not favour high nutrient utilisation (Kiarie and Mills, 2019). Furthermore, and with regard to fibre, different processing methodologies may be used to modify 'gut health' after weaning.

Molist et al. (2009) showed that wheat bran (WB), a fibrous ingredient, could decrease the number of pathogenic *E. coli* in the faeces and reduce the incidence of post-weaning diarrhoea. Molist et al. (2010) extended this study by trying to determine whether the effects were due to WB alone and (or) the particle size. Four experimental groups were tested, i.e., (1) a negative control diet (NC) based on corn, wheat, barley and soybean meal (2) NC + 4% coarsely milled WB (WBc, 1088 μm); (3) NC + 4% finely milled WB (WBf, 445 μm); and (4) a positive control diet (PC) consisting of the NC diet supplemented with a commercial feed grade antibiotic mix. Pigs were inoculated with 6.2×10^9 cfu/mL of *E. coli* $K88^+$ (F4). There were no significant differences in performance attributable to dietary treatment, but the inclusion of WB, either fine or coarse, decreased ($P<0.05$) *E. coli* numbers in the ileal digesta. The use of WBc had an additional benefit because the *E. coli* $K88^+$ numbers were lower ($P<0.05$) compared to WBf (Tab. 1).

Table 1. Effect of wheat bran after 16 days of feeding on the *E. coli* K88 determination (log_{10} CFU/g digesta) in the ileal mucosa and the *E. coli* population (log_{10} CFU/g digesta), and the faecal score in pigs challenged with enterotoxigenic *E. coli* K88 at day 9 of trial (from Molist et al., 2010).

Item	**Diets[1]**				**SEM[2]**	***p* value**
	NC	**PC**	**WBf**	**WBc**		
E. coli K88 determination	4.7^{x}	4.7^{xy}	2.2^{xy}	0.7^{y}	2.66	0.021
E. coli population	6.3^{x}	6.3^{xy}	4.9^{y}	4.1^{y}	2.11	0.014
Total SCFA	22.1^{xy}	20.0^{xy}	14.7^{y}	22.4^{x}	6.10	0.042
	Faecal score[3]					
6 h post-challenge	1.5	0.6	1.0	0.5	0.93	0.157
24 h post-challenge	1.4	0.6	1.0	0.5	0.75	0.066
48 h post-challenge	1.5^{x}	0.6^{xy}	1.1^{xy}	0.5^{y}	0.71	0.025
72 h post-challenge	1.5^{x}	0.5^{y}	1.1^{xy}	0.5^{y}	0.70	0.014
Overall	1.3^{x}	0.5^{y}	1.0^{xy}	0.5^{y}	0.66	0.020

^{x,y} Different superscripts in the same row denotes significant difference ($P<0.05$). [1]Diets: NC, negative control diet; PC, positive control diet with antibiotics; WBf, milled wheat bran diet and WBc, coarse wheat bran diet. [2] SEM: Standard error of the mean. [3]Faecal score: 0, normal; 1, mild diarrhoea; 2, moderate diarrhoea; 3, severe diarrhoea.

Conclusions

The post-weaning 'growth check' and enteric diseases including post-weaning diarrhoea continue to represent a major source of economic loss in some parts of the world's swine industry. The 'gut health' of the young pigs is generally negatively affected, and it can take weeks rather than days for pigs to recover. A healthy GIT should enhance the overall capacity/ability of the host to respond and adapt to challenges/stress and should be concomitant with optimal performance. Much research into the effective uses of feed ingredients, feed strategies and (or) feed additives has occurred to reduce the industry's reliance on antimicrobial compounds. Fundamental to this research must be inquiry into the GIT of the young pig around weaning. A number of nutritional strategies have been suggested as alternative means of enhancing post-weaning growth performance and 'gut health' in piglets.

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Public health impact and control options of parasitic zoonoses via pork

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Introduction

Parasites have long been neglected but nowadays are becoming more recognized as important foodborne pathogens. Various factors contribute to their undervaluation such as complex lifecycles and transmission routes, prolonged incubation period and chronic disease manifestations. In addition, rapid and sensitive diagnostic assays are not always available and therefore parasite occurrences are often underreported, resulting in low disease burden estimates, compared to viral or bacterial causes of foodborne diseases (Newell, Koopmans et al. 2010). In 2015, WHO Foodborne Disease Epidemiology Reference Group (FERG) reported the global disease burden of foodborne diseases expressed in disability adjusted life years (DALYs) of 31 infectious pathogens and chemical hazards (Havelaar, Kirk et al. 2015). Among the highest-ranking etiological agents of these foodborne diseases, many originated from animals (zoonotic pathogens) and included not only bacteria such as *Salmonella* and *Campylobacter* spp. but also foodborne parasites such as *Taenia solium* (*T. solium*) in the top list.

T. solium causing taeniasis and neurocysticercosis, is an important zoonotic pathogen including humans and pigs. It has the highest global disease burden of all parasitic foodborne diseases with 2.8 million DALYs (Havelaar, Kirk et al. 2015). It mainly affects poor communities in low- and middle-income geographic areas, such as in Africa, South America and South East Asia and are indicative of poor hygiene standards of pig husbandry practices. *T. solium* is on the WHO list of neglected tropical parasites, with the high DALY estimation mainly caused by neurocysticercosis (Torgerson, Devleesschauwer et al. 2015). Therefore, control and elimination programmes should be high priority and goals were set for interventions in selected countries by WHO ((World Health and Thomas 2015)).

In addition, two other parasitic zoonoses were also included in the FERG disease burden estimations. *Toxoplasma gondii* (*T. gondii)* causing toxoplasmosis, *Trichinella spiralis* (*T. spiralis*) and other *Trichinella* species causing trichinellosis. Those are both important pathogens that can be transmitted from pigs to humans. *Toxoplasma gondii* was ranked after *T. solium* as one of the highest ranked foodborne parasites (Havelaar, Kirk et al. 2015, Torgerson, Devleesschauwer et al. 2015). Trichinellosis had the lowest disease burden estimated by FERG (Devleesschauwer, Praet et al. 2015), however, it is the only foodborne parasite that is mandatory controlled in many countries for which international guidelines for trade are available (http://www.fao.org/fao-who-codexalimentarius/codex-texts/guidelines/en/). Although it remains questionable whether the low DALYs for trichinellosis are the consequence of these control efforts. In addition, the Codex Alimentarius Committee on Food Hygiene (CCHF) (http://www.fao.org/fao-who-codexalimentarius/en/) became more interested in the importance of foodborne parasites and requested FAO and WHO ' to review the current status of knowledge on parasites in food and their public health and trade impact in order to provide CCHF with advice and guidance on the parasite-commodity combinations of particular concern' in order to support CCHF to develop more general guidelines for the control of foodborne parasites. In 2012, experts from five continents were invited to prioritize foodborne parasites (FBP) using multicriteria decision analysis, an approach often used to rank diseases in a way that comparisons can be made. Of the twenty-four foodborne parasites of global concern, *T. solium* ranked the highest followed by *Echinococcus multilocularis, E. granulosus* and *T. gondii (Robertson, van der Giessen et al. 2013, World Health, Food et al. 2014).* One of the main recommendations was that the regional ranking could be very different from the global ranking and the same exercise should be carried out at the regional level). During a European financed COST Action the European network of foodborne parasites (https://www.euro-fbp.org/) , the same methodology was repeated for Europe. In Europe, *Echinococcus multilocularis* was the highest ranked FBP followed by *T. gondii, E. granulosus* and *T. spiralis* (and other *Trichinella* spp.) (Bouwknegt, Devleesschauwer et al. 2018).). In Europe, the impact of *T. solium* was not clear and studied in another COST action Cystinet (https://www.cost.eu/actions/TD1302).

In order to study the public health impact of these three foodborne and zoonotic parasites, we developed quantitative risk assessment (QMRA) models with the following aims:

1. *Toxoplasma gondii*: to get more insight in the relative importance of meat products for human infections;
2. *Trichinella spiralis*: to compare the public health risks of pigs kept under controlled and non-controlled housing conditions;
3. *Taenia solium*: to assess the risk of human *T. solium* exposure from home slaughtered pigs versus slaughtered pigs with meat inspection.

In this paper we summarize the framework of the QMRA models per FBP and the results. The results can be used to give directions for control options to reduce the disease burden of these foodborne diseases.

Quantitative microbiological risk assessment for three foodborne parasitic diseases

Toxoplasmosis

The life cycle of *T. gondii* entails two main infectious stages: 1) oocysts are excreted in the environment by recently infected felines (definitive host) and become infectious to virtually all warm-blooded animals upon sporulation, 2) tissue cysts develop in muscle and nervous tissues of intermediate hosts and are infectious to felines and other intermediate hosts via carnivory. Thus, consumption of raw or undercooked meat is one of the main sources of *T. gondii* infection in humans. In case a woman is primary infected during pregnancy, the parasite can be transmitted transplacentally and result in abortion, or congenital disease in the newborn. The classical triad for congenital toxoplasmosis consists of chorioretinitis, intracranial calcifications and hydrocephalus, however, often newborns show other less specific symptoms or are asymptomatic at birth. In immunocompetent individuals, the acute phase of the infection usually passes asymptomatically or signs are limited to lymphadenopathy and mild flu-like symptoms. However, both congenitally infected children and immunocompetent individuals that acquired an infection later in life are at risk for developing chorioretinitis. In severely immunocompromised patients (e.g. AIDS-patients or patients under strong immunosuppressive treatment), uncontrolled parasite replication from primary or by recrudescence of a latent infection can result in potentially fatal encephalitis, pneumonitis, myocarditis or disseminated toxoplasmosis. Mainly the lifelong health implications of congenital toxoplasmosis, but also the effects of ocular toxoplasmosis after acquired infection contribute to the disease burden in DALYs (Havelaar, Kirk et al. 2015, Torgerson, Devleesschauwer et al. 2015).

QMRA meat borne *Toxoplasma gondii*

To study the relative contribution of different meat products a quantitative risk assessment model was set up (Opsteegh, Prickaerts et al. 2011). In this model (Fig. 1) data on the prevalence and concentration of *T. gondii* in livestock was combined with data on preparation and consumption of various meat products. Moreover, inactivation by meat processing (i.e. heating, freezing and salting) was modelled and a dose-response model based on data from animal experiments (Guo, Mishra et al. 2016) was used to predict the probability of infection per consumed portion. Despite a low prevalence of *T. gondii* infection in cattle, consumption of beef is predicted to be the main source of *T. gondii* infection via meat in the Netherlands (Opsteegh, Prickaerts et al. 2011, Deng, Swart et al. 2019). Pork products were estimated to contribute 11-12% of meat borne infections. The relative contribution is strongly influenced by preparation and consumption habits. In the Netherlands, fresh meat is generally heated properly and two popular ready-to-eat raw meat products were most important: “Filet americain” (a raw beef spread) and “theeworst” (a raw pork sausage) add up to 90% of the total of predicted meat borne infections (Deng, Swart et al. 2019).

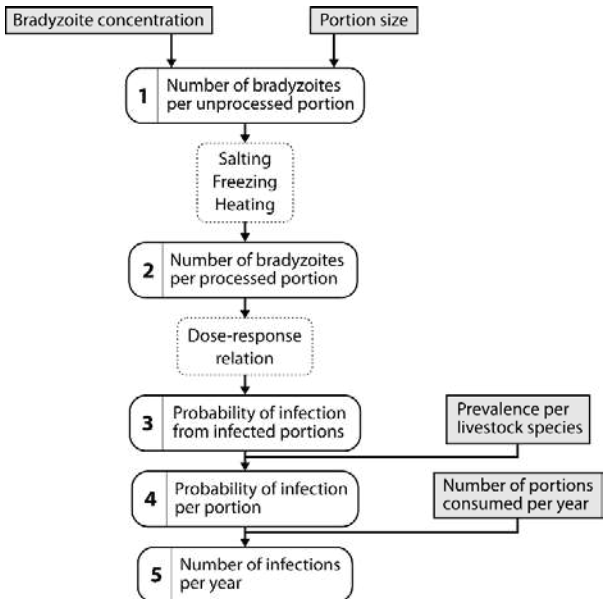

Figure 1. Schematic overview of the quantitative risk assessment model for meat borne T. gondii infections. (From: (Opsteegh, Prickaerts et al. 2011).

The QMRA model developed for the Netherlands was applied for mainland China, using average consumption data per day per livestock species and preparation habits based on American consumers. Here, pork and chicken were predicted to be the most important sources of meat borne infection (Deng, Swart et al. 2019). However, the predicted number of infections was at least thousand-fold higher than estimated based on epidemiological data, which demonstrates the need for detailed and country-specific data on consumption and preparation habits.

Trichinellosis

Nematodes belonging to *Trichinella* spp. can cause trichinellosis in humans. *T. spiralis* has a worldwide distribution and infected pigs are a main reservoir for human infections. Trichinellosis is a serious disease in humans and clinical outcome can vary from mild to very severe. High dose infections can result in acute heart failure and death. Control of *Trichinella* in pigs by the end of the nineteenth century was the start of meat inspection in Europe. Nowadays, control is still mandatory under EU legislation in Europe and many third countries for trade of pork (van der Giessen, Franssen et al. 2013) (Franssen, van Andel et al. 2016). Pigs kept under controlled housing conditions in Europe showed a negligible risk and most outbreaks occur after consumption of meat originating from backyard pigs and wild boar. Therefore, risk based surveillance and control is an option and quantifying human risk can give direction to risk-based control options.

QMRA *Trichinella spiralis*

The QMRA-T workflow started from parasite prevalence and abundance for pigs originating from controlled and non-controlled housing. *Trichinella* distributions between and within pigs were modelled and the outcome was used to estimate the probability to encounter *Trichinella* larvae at meat inspection. From there, probabilities of encountering *Trichinella* larvae in pig portions were modelled and subsequently, the probability to become infected from consuming cooked portions of pork was quantified, using an earlier published dose-response model for *Trichinella* in humans (Teunis, Koningstein et al. 2012). Finally, we estimated human incidence of trichinellosis for the European Union. Moreover, the effect of test sensitivity on human trichinellosis incidence from pigs from non-controlled housing was quantified.

The estimated annual risk from pigs from non-controlled housing was 59,443 human trichinellosis cases without testing at meat inspection and 832 (95%CI 346 – 1410) cases with Trichinella testing, thus preventing 98.6% of trichinellosis cases per year by testing at meat inspection. Using the QMRA-T, already a 5% decrease in test sensitivity resulted in four times higher human trichinellosis cases from this housing type, compared to baseline incidence.

The estimated annual risk for pigs from controlled housing was less than 0.002 (range 0.000 – 0.007) human cases with- and less than 0.010 (0.001 – 0.023) cases without *Trichinella* testing at meat inspection, which does not differ significantly (p=0.2075). In practice, this means no cases per year irrespective of *Trichinella* testing. Thus, controlled housing effectively prevents infection and *Trichinella* testing does not contribute to food safety for this housing type. Not testing for *Trichinella* requires evidence based full compliance with regulations for controlled housing (Franssen, Takumi et al. 2018).

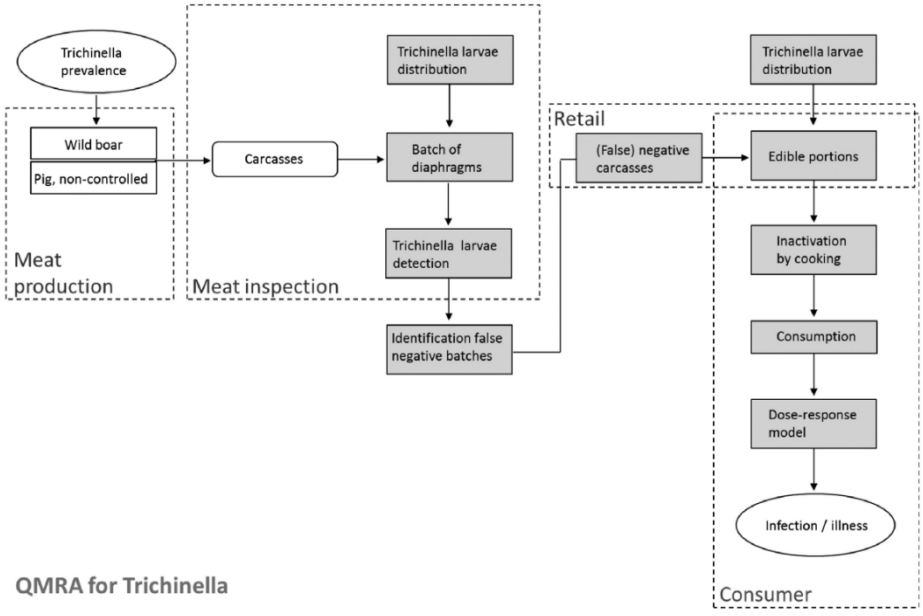

Figure 2. Schematic overview of the quantitative risk assessment model for meat borne T. spiralis infections. (From: Franssen et al., (2017), International Journal of Food Microbiology 241 262–275).

Taenia solium/cysticercosis

Taenia solium is a zoonotic tapeworm with pigs as the intermediate host and humans as the definitive host. The adult stage of the parasite in the human host can cause taeniasis when raw or undercooked meat of infected pigs is consumed. In humans, the zoonotic tapeworm produces hundreds of eggs, which are released in the environment through faeces. Humans can also act as intermediate hosts when colonized by embryonated eggs, either through autoinfection/self-infection or contaminated food or water (cysticercosis). When cysts localize in the central nervous system, it might lead to neurocysticercosis (Trevisan, Sotiraki et al. 2018).

QMRA *Taenia solium*/cysticercosis

QMRA *T. solium*

The aim of this QMRA model was to quantify the risks of human *T. solium* exposure from consuming pork originating from pigs slaughtered at home and controlled slaughterhouses for five European countries (i.e. Bulgaria, Germany, Poland, Romania and Spain) (Meester, Swart et al. 2019). The model took different stages of the food chain into account and comprised three parts, started from production, then inspection, and consumption. Data on the prevalence of porcine cysticercosis, the percentage of home slaughtered pigs, the sensitivity of meat inspection, cyst distribution in pork, and pork consumption amount were collected and used as inputs for the QMRA model (Fig. 3). The question was if consumption of undercooked meat from home slaughtered pigs without meat inspection is a higher risk for humans than pigs under controlled conditions with meat inspection in Europe.

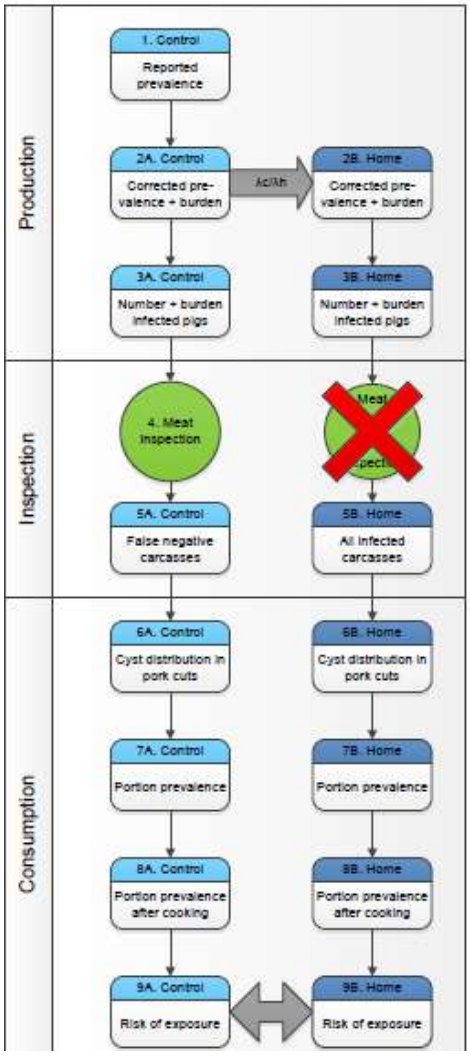

Figure 3. Conceptual risk chain of Taenia solium exposure (Meester, Swart et al. 2019).

At the production part, the model sets off with the reported porcine cysticercosis prevalence and adjusted it with sensitivity of meat inspection. Then, the rate of pigs exposed to eggs and infection loads of porcine cysticercosis in muscles were calculated. In the end of the production part, the number of infected pigs for both home slaughtered and controlled conditions were determined. Since meat inspection is obligatory at slaughterhouses in European Union, the inspection part was included in the controlled condition. At the consumption part, all the false negative/infected carcasses from the two conditions estimated at previous steps were included. And then, the probability of a cyst to enter specific meat cuts, and cyst distribution in consumed portions were predicted. Finally, after applying four cooking scenarios, the prevalence of infected portion originated from controlled slaughterhouses and home slaughtered pigs were estimated.

Results showed that the prevalence of contaminated pork portions from home slaughtered pigs was almost 14 times higher than from slaughterhouses. This was mainly influenced by the prevalence of cysticercosis in pigs. The sensitivity of *T. solium* inspection depended on the infection load of the heart, and each heart cysticercus has a probability of 0.32 to be detected. Although the test sensitivity of inspection was low, it did not affect the risk of exposure. The results of cooking scenarios showed that cooking can effectively reduce the risk of exposure to *T. solium* infected pork (Meester, Swart et al. 2019) .

Discussion and conclusion

Quantitative risk assessment can direct risk-based surveillance and control options. In this paper, we have shown QMRA models developed for three different meat transmitted parasitic infections. In case of *Toxoplasma gondii*, the QMRA models included the prevalence of infection in the main meat producing animal species, concentration of bradyzoites in meat, dose response relationship, meat consumption data and *T. gondii* inactivation characteristics i.e. salting, heating and freezing of meat. The QMRA results showed that beef was most important and pork products contributed to 11-12% of predicted meat borne infections in the Netherlands (Deng, Swart et al. 2020). The relative contribution was strongly influenced by preparation and consumption habits and can differ from country to country. In the Netherlands, two popular ready-to-eat raw meat products were most important (Deng, Swart et al. 2019). This has guided further research towards the effects of meat processing and may lead to intervention measures targeted at these specific products, either by freezing of the meat used for production (Suijkerbuijk, Over et al. 2019) or by adjusting other processing parameters (e.g. salt concentration) to ensure *T. gondii* inactivation. In China, food habits are very different from European food habits and here pork and chicken were predicted to be the most important sources of meat borne infection (Deng, Swart et al. 2019), however human consumption data in China were only available at the livestock level. Therefore, to identify the risky meat products contributing most to human infections in other countries, the QMRA model can be used as long as country-specific food consumption and preparation data are collected using a similar method. A project aiming to study meat borne source attribution just started under the European Joint Programme One Health (https://onehealthejp.eu/jrp-toxosources/). The described QMRA model will be assessed with food consumption data from nine European countries to obtain better insight of control options in Europe.

To evaluate control options for *Trichinella* infections in pigs, a QMRA was developed starting from the risk of wild boar, backyard pigs and pigs under controlled housing conditions. The QMRA model predicted the number of infections in Europe from backyard pigs and controlled housing pigs and results showed that meat inspection for *Trichinella* reduced the number of infections after the consumption of pork from backyard pigs substantially. However, meat inspection had no effect on the risk after the consumption of pork from controlled housing pigs indicating that *Trichinella* control of controlled housing pigs has no additional value.

To assess the risk of *T. solium* after eating pork of pigs originating from home slaughtering in Europe, a QMRA model was developed. The model included two pathways: home slaughtering and slaughtering under meat inspection. Based on the slaughter data of five countries in Europe, it was shown that prevalence of contaminated pork portions from home slaughtered pigs was almost 14 times higher than from slaughterhouses. This was mainly influenced by the prevalence of cysticercosis in pigs. The test sensitivity of meat inspection was however low. The model also quantified that cooking can effectively reduce the risk of exposure to *T. solium* infected pork (Meester, Swart et al. 2019).

In conclusion, we presented three quantitative risk studies for foodborne parasites, and highlighted their utility in aiding the assessment of disease burden and potential effects of interventions. Such QMRA studies need to be carefully designed, and uncertainties present in the model must be explicitly considered. Indeed, major data gaps in the consumption phase were identified, not only frequencies of consumption or portion sizes, but also preparation habits. Also, dose-response relations remain hard to quantify. However, despite remaining challenges, QMRA studies have come a long way since their inception in the 1990s, with significant methodological advances, and have become the the-facto method of choice for microbial risk assessments.

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Imagining a Digital future for Precision Pork Production

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Introduction

Pork's historic position as the world's most popular meat is no more. 2019 was characterized by the explosion of African Swine Fever in China and subsequent slaughter of approximately half of that country's pigs, representing about 25% of the worlds production. Allowing for this catastrophe the exorable rise in the global consumption of chicken ensured it always going to result in its accession to No.1 spot worlds, but the rapid growth in the consumption of seafood, and now of alternative proteins threatens meat producers with market disruption as never before.

Despite cultural and religious exclusions in many societies pork was traditionally the most widely eaten meat in the world, accounting for 36% of all meat consumed, with Asia is the biggest consumer. The continued growth in the middle class, with China's expected to double in the next 15 years, surely heralds a return to favor for pork. Most critically the world's leading producers including China, the US, Brazil, Spain and Russia, face future challenges that could be reduced, or even eliminated, with the implementation of digital and other data driven technologies.

From a consumer (or 'Prosumer') standpoint concerns over animal welfare, the use of ingredients in the feed that they don't approve of (e.g. antibiotics) and the environmental footprint of pork production predominate. The threat of displacement by other meats (poultry, fish) and increasingly plant based, and other protein alternatives is real and the billions of dollar valuation by start-up's as money pours into IPOs demonstrate investor appetite for 'clean' meats. Processors focus on employee safety and welfare priorities even while trying to improve meat production efficiencies. For producers and farmers disease mitigation, animal health and performance are top of mind even as labor shortages on farm dominate conversations in countries all over the world. What is required to address the concerns of all three groups of stakeholders. Can feed conversions be reduced to below 2:1? Can farmers healthily gain more pigs per sow, and keep them alive? How do we maximize meat yield from each carcass? Despite these challenges and the increased need for production efficiencies the farming of pigs has changed little in the last decades, often relying on technologies from the 1970s to accomplish these tasks.

The eight digital technologies framework (PwC) is a useful way to review technologies with the potential to be the most transformative in changing pig production. They can benefit the pig industry in ways that meet these concerns and offer the opportunity for improving processes, increasing productivity and efficiencies while making the animal production process safer for both humans and animals alike.

3D Printing

While the implementation of 3D printing is progressing somewhat slower than some of the other technologies, its value is unquestioned when speed is of the essence and the remoteness of the farm is a challenge. The primary capability of 3D printing is physical items from data received over the net. Imagine the potential to allow farmers to print in minutes parts or pieces for machinery or equipment that break and interrupt the farmer's ability to carry on with daily duties as they wait hours or days for the replacement to arrive. An example could be for suppliers of pig or feed equipment from Germany or China could repair remote on the farm in Africa. Technology such as a 3D printer save time, resources and certainly productivity.

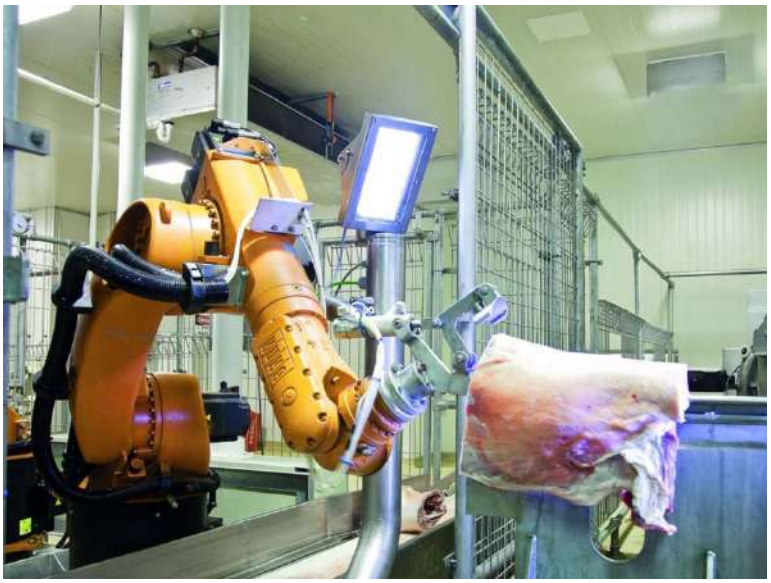
HogSlat is a US based company that is experimenting with the technology to print plastic prototype machine parts using a 3D modeling software system and liquid plastic as "ink." The printer itself is comparable in size to a mini refrigerator and can print a product up to 10"X10"X12" in size. While replication is functional today it can take several hours or possibly even days to create the replacement part. In an urgent situation this may not be feasible but points to the future.

Food printers are now available at an 'affordable' price of $2,000. Using food ingredients today the printing of pizzas and cakes is possible using starches and proteins but with the inclusion of ingredients derived from pork production, printing pork patties, sausage will be possible. Already one company, Meadow meats, has announced a desire to print meats including pork.

Robots

The agricultural robotic industry is expect to reach sales of $8.82 to $15 billion within the next five years. Robots are being embraced in many agricultural sectors including crops, vegetables, processing plants and on farm and anywhere finding qualified labor is a challenge.

Swine Robotics has an entire line of robots designed to improve animal welfare, safety and production. The Boar Bot is a robotic boar which individually check each sow for heat detection and visiting each sow for just enough time to determine her level of interest, improving heat detection and accuracy in insemination and farrowing rates.



Danish consortium SkaldTek pulled together 12 different companies, institutes and universities to build a joint project designed to improve the entire swine production process using improved technologies such as robots and sensors. According to SkaldTek not much has changed in swine production for over 40 years. The consortium's focus is to improve swine barn operations and equipment, address environmental concerns (reducing odor and emissions) and lessen societal concerns (animal welfare).

On the assembly line, robots are replacing many of the more dangerous roles in meat processing and packing. These robots use artificial intelligence and machine learning to create algorithms designed to cut the meat in the most efficient manner. New Zealand's Scott Technologies designs robots for meat processing and pig carcasses and in 2016, the world's leading meat processor, Brazil's JBS, bought a "controlling share" of Scott for $41 million, a clear sign that the world meat producers see this as a critical part of their future.

Ramsta Robotics designed Clever Cleaner, a robot with the sole purpose of cleaning empty swine houses. Company documents explain that a cleaner environment rid of mites, fungal spores and bacteria, will lead to healthier

pig production. The JHminiStro is designed to distribute bedding materials or separate particles such as sawdust and manure, possibly saving up to 30% of bedding materials. It can also distribute feed roughage such as corn silage to sows' Washpower's Procleaner is another robot designed especially for cleaning pig houses.

Robotic pork processing companies in this area include Jarvis who offer a line of several different robots to perform an array of meat cutting tasks and touts its easy to clean machine, the KUKA robot, which demonstrates its skill at meat cutting in a YouTube video.

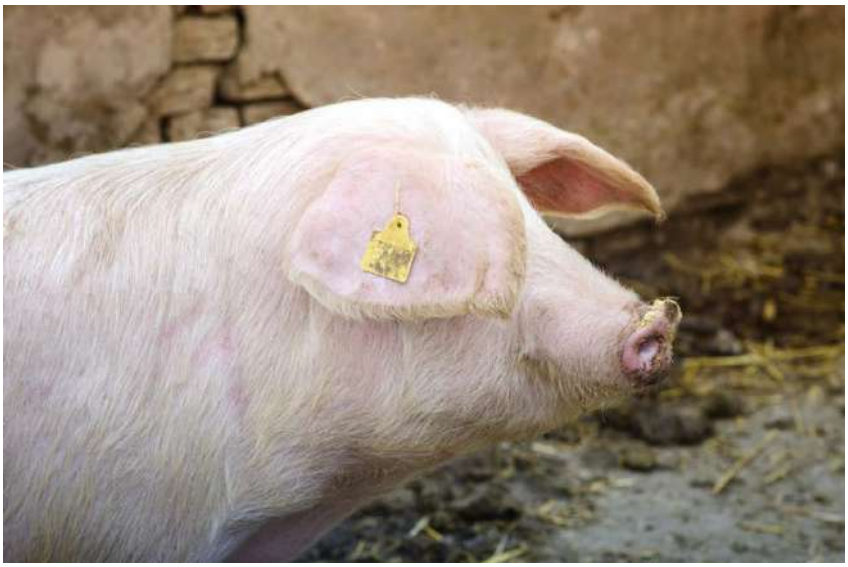
Drones

There is little justification for the use of unmanned aerial vehicles (UAV's) or drones in pig production, although the use of micro drones, smaller than the size of your hand, are growing and might negotiate the indoor environment better without disturbing the pigs. Drones are being used extensively in crops, to scout fields, and potentially, they could be used to scout outdoor herds and check for illness or vices, but other than this, drones do not offer a clear opportunity for pig producers.

Sensors

E-Doctor is the brainchild product of SmartAHC, a Singapore-based startup working its way into China's pork industry. The wearable measures each individual sow's physiological data and combines with artificial intelligence to predict disease risk and ovulation time. With a low energy consumption model, the device can be worn for up to three years for data collection and analysis. ETag, from the same company is fitted to a pig's ear, with no need for human updating or adjustment, remotely identifying pigs and maintaining accurate records, creating an individual report for each pig on the farm.

Another company in the same area is Agrisyst. Ear tag sensors monitor and document information representing the entire life of the pig supplying customers with real time data immediately available for full reports including veterinarians, food suppliers, processing plant reports and data. Financial reports and analysis generated from this can help farmers to procure bank loans and contracts more effectively.



Soundtalks, who have received substantial investment from Boeringher Ingleheim, and Fancom are both using sensor to analyze breathing and coughing sounds to identify respiratory illness and early stage infections. Fancom also produces a system called eYeScan, which uses computer imaging to determine calculate an animal's weight.

Smartbow, an Austrian start-up acquired by global animal health leader Zoetis, has a strong presence in the dairy industry for its sensors and is now demonstrating the use of it technology, to monitor animal activity and movement and detect heat cycles, in pigs.

Until recently, monitoring ammonia in pig houses was nearly impossible. Sensors were overly sensitive to dust, humidity and the gas itself. Big Dutchman's DOL 53 withstands these challenges and correctly monitor ammonia concentration and adjust the ventilation system as needed for pig comfort.

Artificial Intelligence

Given China's twin dominance of the world of artificial intelligence and pork production it is not surprising to see a proliferation of AI dedicated to pig farming applications. These aim to track animals, keeping track of their movement and consumption. Pairing with pig producer, Dekon Group and feed manufacturer, Tequ Group, China's tech giant Alibaba, is attempting to incorporate artificial intelligence and specifically machine vision technology to alleviate many of the difficulties some of these large pig farms face. Cameras are fitted to the ceilings of the barns, allowing producers to individually track each animal using tattooed numbers on their backs. In doing so, they hope to collect data on pigs by maintaining records of breed, age, weight, movement and drinking and eating consumption. Taking it a step further, Alibaba also plans to use voice recognition software to listen to the herd. The system can determine if a pig is falling ill by listening for sounds such as coughing. It can also lower piglet mortality by 3% by listening for squeals of piglet distress, which happens when a mother may be accidentally smothering one of her litter.

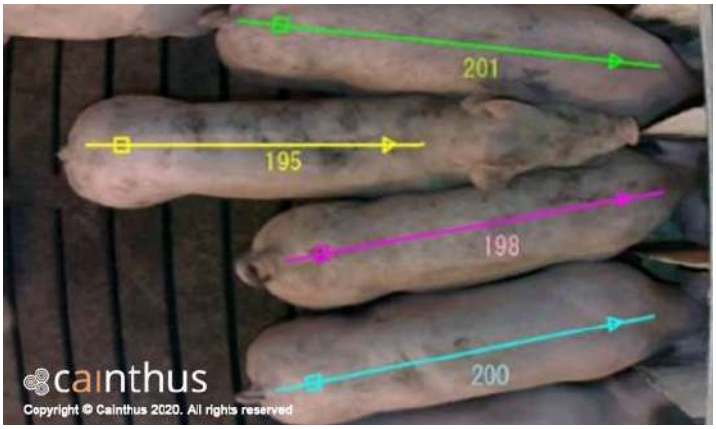
Award winning start-up SwineTech, from Iowa, not only analyses piglets' squeals for distress but also has a technology to prompt the sow to move in order to save the piglet from smothering. The product, SmartGuard, monitors pigs for 24/7 information on vitals, behaviors and biometrics and connects farmers constantly to their herd by providing real time data analysis and already has installations in many farms, notably with integrations in the US.

Harbro Limited, in the UK, has been testing Innovent's qscan, a technology created by scientists at the UK's National Agricultural Engineering Institute, which allows the farmer to monitor weight and growth on a daily basis, and to adjust feed plans based on target growth rates. It also determines which animals falls out the normal range and help producer's, nutritionists and veterinarians' access to management information together, to recognize food, water and health concerns before they become more serious.

Also demonstrating machine vision/AI technologies is a company called Clicr Technologies. Having seen success on Alligator farms their goal is to weigh animals comfortably without human intervention. The precision of the technology improves overall efficiency by allowing farmers to mark animals that are at the ideal shipping weight, reduce feed costs when animals do meet the target weight, administer medications accurately and in a timely manner and eliminate underperforming animals or the sows that produce them.

Tail biting is a common concern among pig farmers. It causes stress, pain and sickness for bitten pigs which can result in economic losses for producers. Tail docking can sometimes reduce tail biting, but in some places, such as the EU, it is viewed as mutilation and is banned from an animal welfare perspective. In order to alleviate tail biting issues Scotland's Rural College is using technology such as machine vision and 3D cameras to detect tail posture and alert farmers when there is potential for increased tail biting.

Cainthus (who I have been working as CEO) is using Camera vision technology to track individual consumption, water intake and other health metrics. What makes them different is how they look at the data. A swine facility is a hazardous work environment for electronics so it's taken some work to get the models to run on camera. Recently backed by a substantial investment by Cargill, Canthus has primarily focused on developing products for Dairy, but has already a development team dedicated to observation of pigs. Cainthus success has been built on the back of the founder's background in farming and agribusiness and working closely with leading producers to design solutions that are practical on farm, and with insights the producer's value.



Augmented Reality

Opportunities for augmented reality in the pig industry could be in assembly line work during the production process itself, determining food safety or as a training tool for producers or processors.

A real world application is the collaboration between food industry technology company, EyeSucceed,

Google's Glass, and NSF International in creating a headset that enables users to communicate back and forth both audially and visually so that the remote observer can experience what the headset user does. It can record video, or even be placed on equipment for a safer view of how a system or operation is working. Augmented reality allows the headset to show the viewer documents, checklists, etc. for easy reference with applications for auditing and inspecting, allowing farmers and producers to follow the process remotely and in testing, inspecting and certification (TIC).

This technology will be useful as a training tool for line workers in a slaughterhouse or food processors to identify issues the human eye cannot see. Unfortunately, since the technology isn't cheap implementation has been slow.

Virtual Reality

Britain's Agriculture and Horticulture Development Board's ADHB Pork has demonstrated at its Stockperson Pro East program a display using virtual reality to demonstrate pig pen ventilation systems. North Carolina's Pork Council brought a three-minute, 360-degree tour of hog farming, to the NC State Fair The video takes viewers through visual representations of corn harvesting, discussing the nutrition requirements of a pig, the inside of a sow barn and finishing barns, discussing how the operation works and answers questions such as why pigs are kept inside.

Virtual reality has been used as a tool to train veterinarians, specifically at the University of Liverpool in livestock such as Horses and Dairy. Many opportunities clearly exist to do the same with pigs.

Blockchain

Blockchain is described in depth in many articles but for the sake of simplicity it is an online documentation system which allows you to hold records of transactions in distributed ledgers maintaining confidentiality for all of the people involved in the transaction. Multiple applications exist for its use in the food and agriculture industry, since it offers traceability and increased food safety, better payment systems, lower costs of production and logistics and opportunities for new ventures and business growth.

Walmart in combination with IBM and Tsinghua University is testing the technology in the pork industry. Pork is the most popular meat in China and often produced in small 'backyard' family farms blockchain can allow tracking information for every package of pork, its movement from producer to consumer can be recorded and offer complete traceability.

OwlTing started a blockchain service for the pork industry called OwlChain last year in Taiwan. The company says its aim is to give consumers faith in the food system again and to guarantee food safety and traceability by verifying claims and certifications.

The US Federal Safety and Inspection Service (FSIS) has recently proposed amendments to regulations for pork meat inspection requiring hog slaughter establishments to provide a public health protection system and verify sanitary standards. While blockchain is not specifically cited, it is the primary technology capable to verify quality and authenticity throughout the entire pork production process.

Internet of Things

Precision agriculture is practically a buzzword in agriculture these days. In order to grow plants, feed animals, produce food with minimal inputs cannot be achieved without considering the implementation of several if not many of the 8 digitals all working together to maximize efficiencies, reduce errors and increase profits. This is often referred to as smart farming. Connected devices or the Internet of Things (IoT) that makes this possible.

EU funded project ALL-SMART-PIGS is making strides to demonstrate the value of IoT in the swine industry. Using sensors to detect health, food and water intake, mobility, weight, air quality and even coughing. As with this program and indeed any others the use of IoT can be limited by internet access on the farm and this can be a challenge, especially in rural areas.

PigVision, out of the Netherlands, offers a full line of pig-specific data for real time reporting, documentation and analysis. Information is stored securely and can be transferred to users' smart phones via the Smart Pigs mobile extension. Maximum Ag Technologies offers an all-around technology solution for producers enabling them to track in real time animal feed and water intake, control temperature and ventilation, set alarms for text or email, all of which is accessible via smart phones, tablets or computers.

Big Dutchman's BigFarmNet applies IoT to connect its customers' controllers, computers and sensors and allow them all to communicate with one another. Farm managers can view 3D images of the farm, evaluate climate conditions and feed settings and even change these settings all within the software platform. None of these applications would be possible without the interconnectivity that IoT allots us.

Nutrigenomics

The influence that nutrition has on genes is reflected in the sciences of nutrigenomics, epigenetics and metabolomics. In general, all of these sciences are limited by the same challenges – an overwhelming amount of data which requires analysis and interpretation. Alltech, natural feed and feed additives has lead investments and publications in this field. The challenge for nutrigenomics is the ability to interpret the data, generate insights and act upon these in a timely manner.

Brazilian company Agriness has come to dominate its home market with an App that makes sense of all of the data sources, interprets them and generates specific actions that the producer can use to maximize performance in the barn and profitability on the farm and today claims 90% of Brazil's pig production is on their system.

Summarizing in order for global pig production to respond to the consumer, environmental and legislative demands on its producer's mush seek to disrupt and revolutionize their process. This will require the implementation of multiple technologies (among the eight digital ones and more) and the ability to interpret the information they generate to create actions that result in improved management practices, production efficiencies and better offerings of pork and pork products that meet every increasingly enhanced consumer expectations.

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Gilt reproductive challenges for the next decade – measuring and managing what we already know

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Introduction

In a recent detailed review of the factors determining reproductive development in the gilt (Patterson and Foxcroft, 2019), we suggested that "Substantial evidence supports successful management of gilts as an absolutely necessary component of breeding herd management and the pivotal starting point for the future fertility and longevity of the breeding herd". We also discussed the link between earlier sexual maturation and improved sow lifetime productivity (SLP). A comprehensive gilt management program will address several key factors that pre-determine SLP, including: Birth traits that determine the e□ciency of replacement gilt production; e□ective selection of the most fertile gilts for entry to the breeding herd; programs that provide a consistent supply of service eligible gilts; and appropriate management of weight, physiological maturity, and a positive metabolic state at breeding. Good gilt management can largely resolve the existing gap between excellent genetic potential and the more modest sow lifetime productivity typically achieved in the industry.

The purpose of this presentation is not to repeat the detailed information presented in our recent review, nor to discuss the detailed physiological mechanisms involved. Rather, we will identify the key components of a good gilt management program in commercial practice and the excellent SLP that can result from applying such programs successfully. We will then look at two key factors that determine the timing of the final gilt selection program. Firstly, the age at which puberty is triggered in gilts and the positive relationship between earlier puberty and SLP. Secondly, data on the growth performance of contemporary gilt populations, highlighting the risk that high growth performance represents for gilt being too heavy at breeding. Next, we will briefly highlight the importance of pre-breeding management in the gilt and the need to create a gilt management program that allows the majority of gilts to be bred at second estrus. By routinely implementing a Heat-No-Serve (HNS) strategy producers can improve first parity performance in gilts, which itself seems to be predictive of SLP. Finally, we will identify those measures of gilt performance for which records are considered to be "non-negotiable" and describe how these records can be used to monitor gilt performance and compliance with established protocols.

Although many other factors can affect the performance of the breeding herd, data-based evidence that an effective gilt replacement program is in place will already allow production systems to meet existing industry benchmarks for excellent SLP.

Key components of an effective gilt selection program

In subsequent sections of this review we will discuss the key factors that determine the actual timing of the final gilt selection program. However, from the outset we wish to establish the benchmarks against which alternative strategies should be measured. In our minds, the exposure of immature gilts to the full spectrum of puberty-inducing boar stimuli continues to be the most effective way of identifying pubertal estrus. A HNS record is the only acceptable way of identifying a "select" gilt and a fully integrated puberty induction program as shown in Figure 1 is the only effective way of recording a HNS event in the 80% of select gilts that can also be bred before 230 days of age.

As shown in Figure 1, this system involves a purpose designed boar stimulation area (BEAR) that allows for: 1) excellent detection of a standing heat in response to signaling pheromones from a team of boars using initial fence-line contact. The record of a HNS event can be simply linked to a weight using either scales or other simpler but reliable measures of weight, like a weight-tape. Gilts are then provided direct contact with a rotation of mature, high libido, boars by releasing one of the available boars into the group of gilts. A minimum of 10 minutes of direct contact, with a gilt:boar ratio of not more than 12:1 in an appropriately sized pen, allows the gilts access to the primer pheromones secreted in the salivary "froth" of the boar. Stockmen frequently fail to realize that the gilt is driving the program here and during the pro-estrous period gilts will actively and aggressively "solicit" access to the boar's salivary secretions. During this same period of direct boar contact, other boars are also providing a priming function thought fence-line contact with the gilts in the group.

In our experience, the use of such BEAR facilities allows the detection of a naturally (boar) induced pubertal estrus in up to 80% of the gilt population within a 30-day final selection program. As discussed later, if a lower percentage of gilts than expected have a HNS event by day 23 of the program, intervention with exogenous gonadotropin treatment is used to elicit a hormonally-induced estrus, as required to meet gilt breeding targets. Both the need to identify the most sexually mature gilts in the population, linked to the problems of older and overweight gilts being presented for breeding if the stimulation period is extended, determines that the BEAR system is essentially run as an all-in/all-out facility. Any gilt not in heat by day 30 is designated as non-select and shipped to market, vacating the space needed for the intake of pre-select gilts to the GDU.

Our belief in the value of this rigorous program of gilt selection is based on evidence of the excellent lifetime performance of the gilts delivered to the breeding program, accepting that the timing of the selection process allows for all gilts to be bred at least at second estrus. Figure 2 shows the results of a study carried out in collaboration with a commercial production system in North America, in which 288 gilts were entered every four weeks into an off-site gilt development unit (GDU) with duplicated BEARs facilities, that allowed a 28-day period for acclimation, vaccination etc., before implementing a 28-day period of stimulation with boars (Patterson et al., 2015).

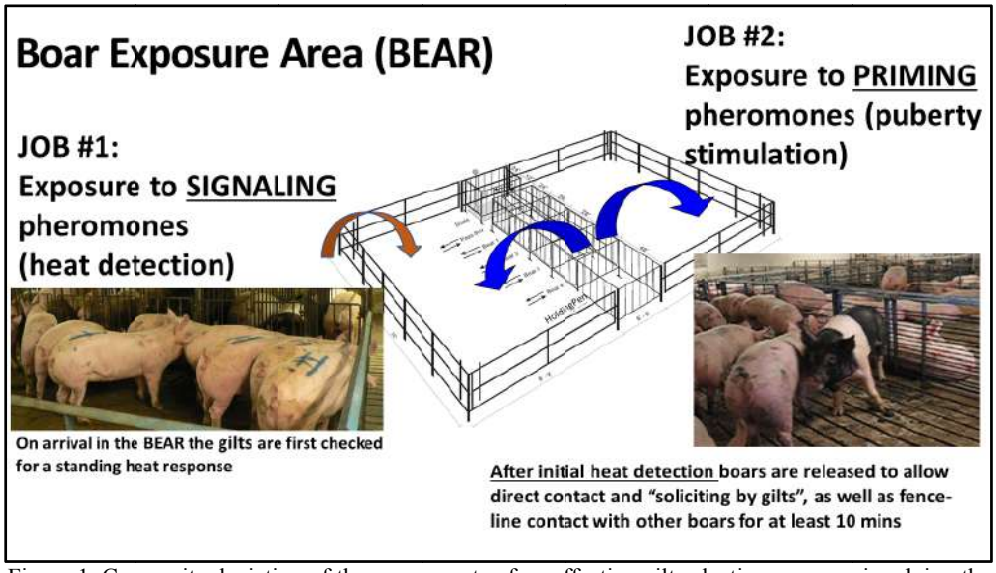

Figure 1. Composite depiction of the components of an effective gilt selection program involving the exposure of successive groups of gilts to both fence-line and direct contact with boars using a purpose-built boar exposure area (BEAR).

	Classification	
	NAT	**PG600**
Nos. delivered to sow farm	2,374	741
Nos. (%) served of delivered	2,318 (97.6)[a]	709 (95.7)[b]
	% gilts delivered	
RATE	NAT	PG600
P1 Pregnancy rate (first serve)	96.3[a]	93.8[b]
P1 Farrowing rate (first serve)	94.7[a]	92.0[b]
P1 Farrowing rate (multiple serves)	96.3[a]	94.1[b]
Retention rate at P2	87.6	85.0
Retention rate at P3	79.2	76.7
Retention rate at P4	70.6[a]	65.3[b]

Figure 2. Retention rates until fourth parity of select gilts delivered from an off-site GDU and designated as select on the basis of a natural (NAT), boar-induced pubertal estrus, or a pubertal estrus induced with exogenous gonadotropin injections (PG600) after 23 days of exposure to boar stimuli. Data derived from Patterson et al. 2015 . [a,b] Different superscripts within the same row indicated significant differences at $P < 0.05$.

During this particular trial period, select gilts with a HNS record were moved to a down-stream sow farm every three weeks and 76% of the select gilts had shown natural, boar-induced, puberty, with the balance of gilts having a recorded HNS event in response to gonadotropin (PG 600) treatment (see Figure 4 for typical responses). The pre-breeding protocol stipulated that gilts were given at least 14 days of acclimation to stall housing before being bred at either their second of third heat. As can be seen in Table 1, the retention of these gilts within the breeding herd exceeds all benchmarks set by the industry, with relatively little difference in performance between natural- and PG 600-induced estrus. However, it must be appreciated that these hormone-induced estrus events were triggered in gilts that had already been exposed to 23 days of boar primer pheromones, which has been shown to significantly increase the response of gilts to exogenous gonadotropins. As well as the excellent retention in the sow herd, these gilts produced over 55 total pigs born over their first four parities, which generally exceeds the average total lifetime production of the sows in many commercial herds.

The purpose of this first section is to validate the use of proven puberty-induction programs to deliver gilts to the breeding herd with the potential for excellent SLP. The investment in good gilt management and rigorous gilt selection programs seems to largely remove many of the issues with SLP performance frequently identified as issues in the industry. In particular, these programs seem to largely remove problems with gilts that are on inventory but never produce a litter, and gilts that are culled before being rebred after weaning their first litter. In the next section we will try and provide evidence that these outcomes are largely due to the selection of the most sexually mature gilts available and proven links to later reproductive performance.

Age at puberty and lifetime performance

The literature supporting a link between early age at puberty and superior lifetime reproductive performance is extensive and has again been recently reviewed (Patterson and Foxcroft, 2019). For the last 25 years, successive studies have reported that gilts with an earlier onset of pubertal estrus have better overall fertility in terms of pigs produced and lifetime productivity. Additionally, all good managers know that the detection of standing estrus in the sow is the fundamental event that allows our breeding programs to succeed. Therefore, evidence that the "strength" of the behavioral traits at pubertal estrus are associated with the strength of estrus expression in the sow, suggests that a record of full standing heat at pubertal estrus should be an absolute requirement for gilts entering the breeding herd.

In terms of pubertal age *per se*, two questions are important. Firstly, what is the preferred age range that is linked to superior SLP. Secondly, having established this range, how can gilt selection programs be set up to identify these gilts. However, an even more fundamental question to address first is "what is the age of puberty in the gilt?" The answer to this question can only be answered by studying pubertal onset in gilts that are not subject to any constraints to their sexual maturation and then expose these gilts to potent boar stimuli from an early age. When this has been possible, it appears that the most precocious gilts probably show pubertal estrus as early as 130 days, with a fairly normal distribution in age at puberty thereafter. Interestingly, age at sexual maturation in the most precocious gilts has not changed for the last 50 years, and the two biggest variables that determine age at pubertal estrus in commercial production are the age at which puberty induction stimuli are provided, and the efficacy of this stimulation process. Fortunately, in a recent large scale study in commercial facilities, but using high health status gilts and daily exposure to a rotation of mature boars from 160 to 260 days of age, Calderon Diaz et al. (2016) provided outstanding data on which to base decisions about puberty induction programs. In an earlier review of these results, Valet (2015) provided evidence that three different sub-populations of gilts were represented in the overall response profile (Figure 3).

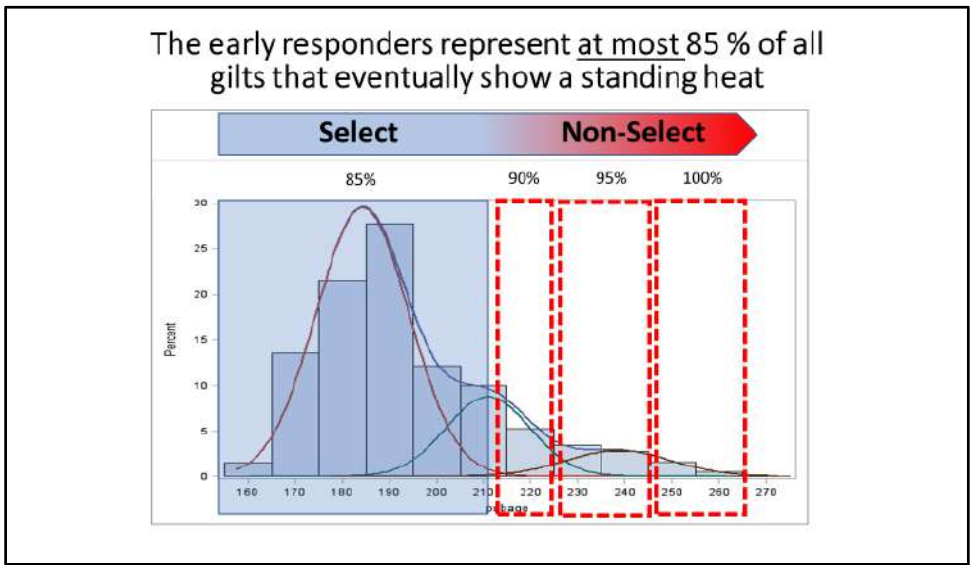

Figure 3. The response profile showing the percentage of gilts reaching pubertal estrus within successive 10-days periods in response to daily stimulation with mature boars from 160 to 260 days of age. Approaching 100% of gilts showed estrus within the 100-day period but distribution analysis identified and initial response profile involving the 85% of gilts that were recorded in pubertal estrus between 160 and 210 days of age. The remaining 15% of gilts seemed to be part of a different response population. From Valet, 2015.

We can consider these response curves in the light of results from studies that link differences in either age at puberty or age at first service, to other measures of reproductive performance. The overall consensus from the published literature (see Patterson and Foxcroft, 2019) is that the gilts that have the least gilt non-productive days, the best retention in the breeding herd, and the best SLP, are those that either reach puberty at less than 200 days or are first bred at less than 230 days. In the latter case, assuming that a HNS program is in place to maximize first litter performance, this again determines that gilts need to be no more than 200-210 days at pubertal estrus. As seen in Figure 3, the population of gilts that are predicted to have superior reproductive performance are those that fall within the first response wave when boar stimulation was applied at 160 days. Therefore, restricting our definition of "select" gilts to those that show a pubertal heat by 200 days of age seems to identify those with the best potential reproductive performance. Having made this determination, the next practical questions are: How limited can the selection program be and at what age should it be implemented? Assuming that the full complex of boar-derived stimuli is provided on a daily basis using mature and high libido boars, our experience is that boar stimulation from 170 days of age can identify most of the truly select gilts within a 30-day period (Figure 4). These response curves are derived from the same GDU/selection program shown in Figures 1 and 2. Across multiple cohorts of gilts flowing through the program, more than 75% of the gilts overall had a recorded HNS event by 200 days of age.

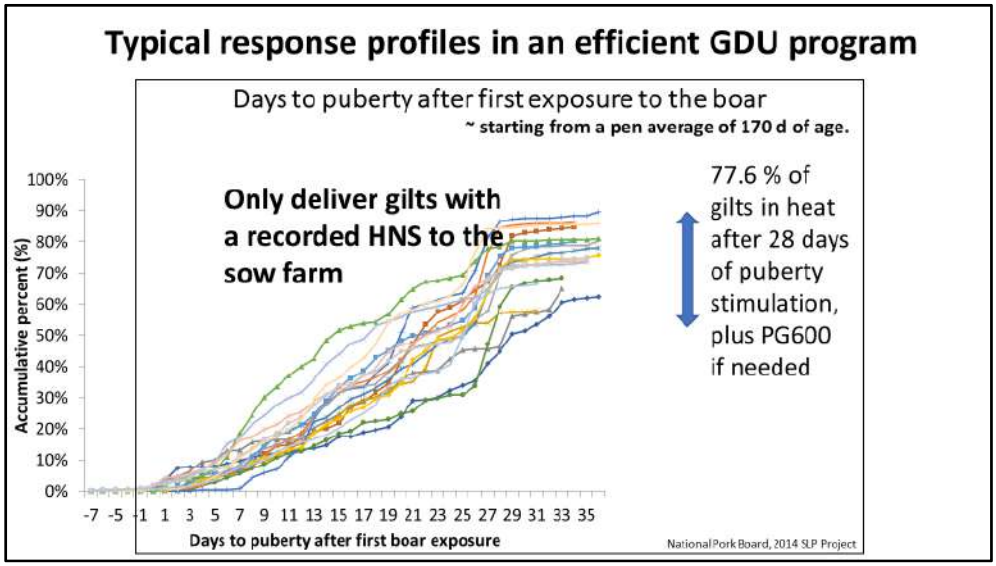

Figure 4. Puberty onset response curves for successive cohorts of pre-select gilts induced to reach pubertal estrus with daily boar stimulation from 170 days of age, with PG 600 treatment of non-pubertal gilts after 23 days of boar stimulation, as needed.

As seen in Figure 3, from the perspective of identifying the first early response group as being the preferred select population, any extension of the selection program beyond 200 days results in later maturing gilts falling in to the select population, with negative consequences for SLP. Starting the program later than 170 days but still ending at 200 days may allow most of the same gilts to express their true pubertal estrus, but optimal use of boar stimuli would be essential. However, if the length of the stimulation/selection window becomes less that 23 or 24 days, there is a possibility that previously pubertal gilts will not be observed in second estrus during the selection window and will be wrongly designated as non-select. Therefore, only taking account of the potential range of pubertal age in the gilt, the preferred selection window that allows discrimination between earlier and later responding gilts, and an adequate period of stimulation and recording to achieve at least an 80% response rate, seems to be 170 to 200 days of age. If less effective stimulation protocols are applied, an earlier start to the selection program, rather than an extension beyond 200 days is preferable.

Although retention of non-pubertal gilts within the gilt pool for more extended periods is common practice, and is justified on the basis of higher selection rates, on balance we suggest that this is a counterproductive approach and comes at a cost. Gilts that take longer to respond to boar exposure have longer entry to service intervals and accumulate excessive non-productive days. The management of these later maturing gilts also involves increasingly inefficient use of labor and space (measured as the number of gilts with a HNS record for each day in the selection program). Most importantly, these later maturing gilts have poorer reproductive efficiency over their productive life and are at risk of being over target weight and having poor retention in the breeding herd. Our preference, therefore, is to run the GDU as a strict all-in/all-out facility, and only allow gilts displaying a full standing heat within the designated selection window to move to the pre-breeding area.

Impacts of growth rate on gilt selection programs

The impressive growth performance of contemporary gilt populations has important implications for the pre-breeding management of gilts. In unselected gilt populations, or when feed restriction is limiting gilt growth and development, it has been possible to show that a threshold growth rate of around 0.55 kg/day exists, below which age at puberty in the gilt will increase. Two recent large-scale studies have confirmed that less than 10% of gilts fail to meet this growth threshold. Indeed, as shown in Figure 5, an increasing proportion of gilts have growth rates at the time of pubertal estrus that place them at risk of being too heavy if bred at second estrus. The scatter of data points in Figure 5 also shows that, as expected, growth rate is independent of age at puberty, and age has no predictive value for determining either age at pubertal estrus or gilt weight at a fixed age. Therefore, in gilt management programs it is essential that both these variables be recorded. Age at puberty (a HNS event at less than 200 days) is used to determine the relative sexual maturity and reproductive potential of the gilt: Weight at HNS is used to manage predicted weight at breeding.

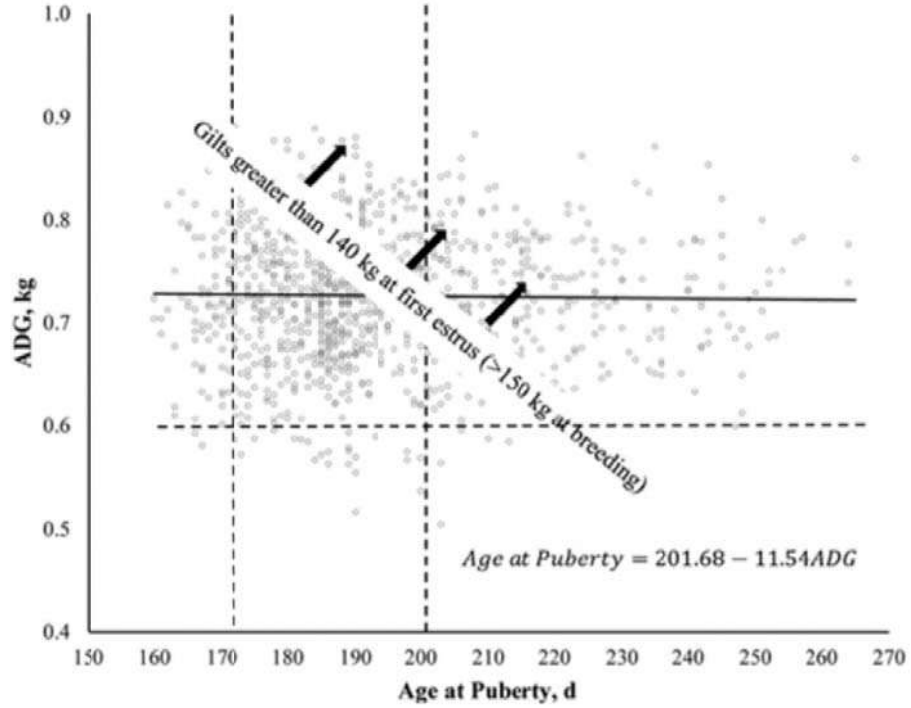

Figure 5. Data from the study of Calderon Diaz et al. (2015) showing the relationship between lifetime average daily gain (ADG) to, and age at, pubertal estrus during a program of daily stimulation of high health status gilts with a rotation of mature boars. The dashed horizontal line indicates the threshold of ADG below which growth would be considered limiting for the expression of pubertal estrus. The solid near-horizontal line shows the fitted relationship between ADG and age at puberty, confirming that these are almost independent variables, with the extremes of growth rate having little overall effect on pubertal age. The extremes of ADG result in gilts with the same age at sexual maturation having a 40 kg difference in body weight. The vertical dashed lines indicate the window of gilt age at puberty that is most consistent with identifying the first wave of earlier responding gilts shown in Figure 3, whilst avoiding serious issues with gilts being too heavy at breeding at second estrus.

Considering again the optimal gilt selection window, we can see from Figure 5 that most gilts with a growth rate between 0.6 and 0.72 kg/day at the time of puberty could be bred at second estrus without exceeding an upper target breeding weight of 160 kg. However, gilts with the higher growth rates are at an increasing risk of being too heavy at breeding if they exhibit pubertal estrus on the later part of the selection window and are still management using a HNS strategy. One response to this risk of being overweight at breeding would be to designate the later responding but faster growing gilts to be bred at first, pubertal, estrus. However, this then prevents the producer from taking advantage of the important pre-breeding management protocols discussed below that maximize first litter performance and SLP. In the absence of technologies to limit growth performance in gilts, perhaps the best compromise is to accept that a population of heavier weight, but select gilts, will exist and that this may have consequences for poorer retention in the breeding herd. If nutritional approaches can be used to limit the growth performance of the faster growing gilts this would be an advantage. However, care should be taken to insure that gilts are returned to a very positive metabolic state in the period preceding puberty stimulation and before breeding.

The main conclusion from this consideration of the impacts of growth rate on gilt management programs, is that exceeding 200 days as the cut-off for identifying select gilts has major consequences for weight at breeding. This second constraint leads us to again conclude that a selection window from 170 to 200 days of age is appropriate.

Pre-breeding management of gilts

Several different groups over the last 20 years have reported a clear relationship between either first or second litter size and SLP, of which Figure 6 is one example. These relationships seem to imply that high performing gilts become high performing sows, and vice versa. Although this fits well with the concept that early maturing gilts will also have better lifetime performance, the biology underlying the predictability of performance shown in Figure 6 is still unclear. Regardless of the mechanisms involved, these relationships suggest that optimizing first litter performance may have ongoing benefits in terms of SLP.

What factors contribute to good pre-breeding management in the gilt? Earlier studies demonstrated that maintaining a high level of feed intake between first and second estrus in the gilt was critical for increasing ovulation rate at second estrus and thus increasing potential litter size born. It seems from studies in more contemporary gilt populations that similar increases in ovulation rate between first and second estrus are not as critical: However, with ovulation rates of 18 in contemporary gilts compared to 14 or less in earlier studies, ovulation rate *per se* may be less of a constraint for first litter size than previously reported. In a more general sense, a number of production-based studies reported an improvement in litter size in gilts bred at second vs first estrus (see Patterson

and Foxcroft, 2019). These trends have been confirmed in more recent analyses of production data and support the concept of building a HNS strategy into the final phase of the gilt development program.

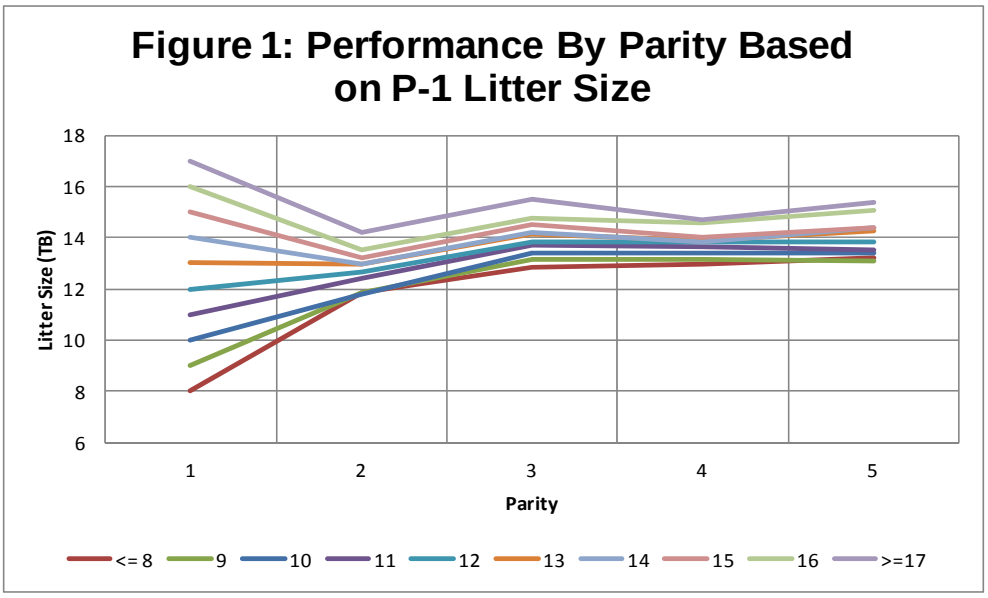

Figure 6. Data demonstrating that first litter size is predictive of total born in later parities in commercial sow populations. From Pinilla et al. (2014).

However, as earlier mechanistic studies reported an immediate and negative impact of reducing feed intake in pre-pubertal gilts on tonic LH secretion, it is important to recognize the sensitivity of the hypothalamic-pituitary-ovarian axis to a reduction in nutrient intake. These potential detrimental effects of reduced feed intake on the reproductive system during the critical period before breeding was part of the rationale for ensuring that replacement gilts have adequate time to properly acclimate to changes in location and housing before being bred. Although a recovery period of at least a week from "acclimation stress" was suggested, leading to a recommendation of 14 days of acclimation before breeding, more recent data suggest that nutritional regimens during the entire 21-day inter-estrus interval may have critical effects on first litter performance. Collectively, the information available suggests that implementation of a HNS program as a routine part of gilt management is important and allows producers to optimize first litter performance.

Measuring and managing critical components of gilt development

In their review of factors that affect the productivity of the breeding herd, Koketsu et al. (2017) stated that "*Gilt development and management is critical to optimize the lifetime reproductive performance of sows. However, even though recording the age of gilts at first estrus and the dates of heat-no-serve can help improve gilt development and management, they are rarely recorded in commercial herds in North America*".

In comparison to the plethora of reproductive data that are routinely recorded for individual sows in the breeding herd, critical data on gilt development is definitely lacking and are not usually a component of the commonly used record analysis programs. As a consequence, the opportunity to objectively evaluate and monitor gilt development programs is lost: Yet good gilt performance is tacitly reported to be critical to future herd performance. Figure 6 identifies the key data that need to be collected to allow effective analysis and trouble-shooting of gilt development programs and shows how this links to the key objectives of a gilt development program. Once gilts receive a unique ID at the pre-selection stage (around 140 to 160 days in most systems) and enter the selection and pre-breeding program, daily records of performance are critical. These data should include estimated body weight at HNS and accumulated non-productive days (NPD) from entry into the herd data base until being successfully bred. It is interesting that the substantial cost of NPD accumulated by gilts that receive a herd ID but never produce a litter seems to be a minor concern to production managers, compared to say a marginal increase in the weaning-to-estrus interval in sows. However, both are indicators of important aspects of reproductive performance that need to be evaluated.

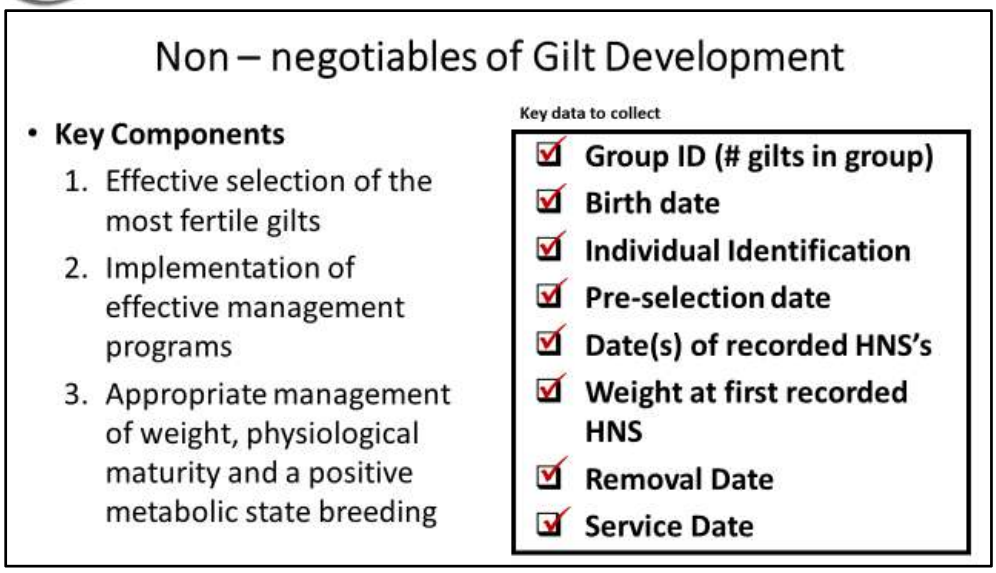

Figure 6. Key data that should be recorded as an integral part of gilt management programs.

Data on gilt performance should not be captured in a vacuum, but should be used to provide weekly updates to production units on the performance of their GDUs relative to other GDUs in the production system. The analyses should also benchmark performance against the performance targets set for the gilt selection programs established. Key outcomes from these analyses could include: Response dynamics of successive cohorts of gilts to puberty-induction programs (as shown in Figure 4); Reports on the proportion of gilts completing a HNS protocol; Plots of the recorded inter-estrus interval (days between HNS and date of breeding); Data on weights at HNS and estimated weights at breeding. Each of these analyses can be used to confirm protocol compliance and to trouble-shoot issues that are affecting performance. Given that a successful gilt replacement program can become the foundation for excellent SLP, one could argue that more emphasis should be placed on the capture and analysis of a limited amount of gilt performance data than on the many metrics of sow performance that are routinely captured. One thing is certain, if the gilt program is badly managed, there will be many negative impacts on downstream sow performance that will need attention.

Conclusions

Establishing an effective gilt development and selection program takes commitment, the allocation of dedicated facilities, and committed staff time. There are no easy short cuts to an effective gilt selection program and this is definitely not "low hanging fruit". However, the impact that well managed gilt development program can have on overall SLP is indisputable. Many of the ongoing issues with sow longevity in the breeding herd that use up valuable resources, but still prevent systems from meeting production targets, can be pre-empted by an effective program of gilt management. The proactive approach of committing resources to good gilt development seems to be a more predictable and less stressful option.

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Vaccines and vaccination in PRRS virus control

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Introduction

The emergence of *Porcine reproductive and respiratory syndrome virus* in the 1980s and early 1990s was a turning point in the ways in which diseases were dealt with in pig farms. The impact of the disease, its rapid spread worldwide (by 1993 the infection was present in America, Europe and Asia) and the unusual features of the virus made evident that a vaccine was urgently needed to try to control the infection. The first vaccine commercialised was Cyblue® an inactivated vaccine produced by Cyanamid and launched in 1993 in Spain. The vaccine offered a limited protection and had the disadvantage of being produced in primary macrophage cultures. Soon afterwards (June 1994), Boehringer Ingelheim launched the first modified live vaccine against PRRSV (PRRSV2) under the name of RespPRRS (USA) and Ingelvac PRRS MLV (worldwide). Next was Porcilis PRRS (Intervet), a PRRSV1 MLV. From that point on, several MLV and inactivated vaccines have been marketed.

What can PRRS vaccines offer?

The first consideration when answering this question is that immunity against PRRSV is only partial –non-sterilizing- against heterologous strains. In practical terms, most, if not all, of the challenge situations that a pig can confront in the field are heterologous challenges. Therefore, vaccinated farms can be infected. What are then the benefits of vaccination? Firstly, vaccinated animals usually show less clinical signs and suffer less complications than unvaccinated ones. For example, abortion rates or the proportion of stillbirths are lower in vaccinated sows compared to non-vaccinated ones (Scortti et al., 2006). The protection against respiratory disease is less evident although vaccinated piglets often are less prone to suffer overt disease and bacterial complications. Secondly, vaccination reduces the duration of the viremia if infection occurs, decreasing the probability of transplacental infection and helping thus to stabilize the herd. Also, at least for PRRSV1, vaccination reduces the transmission by the horizontal route among piglets (reproduction rate below 1) (Pileri et al., 2015). Protection is thought to be mediated by both neutralizing antibodies and by cell mediated immunity. While in most cases neutralizing antibodies are isolate-specific, there are reports indicating that broadly neutralizing antibodies, namely, antibodies capable to neutralize a vast array of strains exist (Martínez-Lobo et al., 2011, Robinson et al., 2018). How and when those broadly neutralizing antibodies are developed is not well known. It probably depends on the strain or strains with which the animal have had contact but also depends on the maturation of the immune response and the repertoire of B-cells in the individual. Regarding cell-mediated immunity, evidences also indicate that there is cross-reactivity between genetically diverse isolates although the epitopes involved have not been elucidated with precision. Unfortunately, at present it is impossible to forecast what level of protection a given vaccine can produce against a given isolate and thus, selection of one commercial brand or another cannot be done based on a prediction of its efficacy for a given farm.

Types of vaccines and vaccination schedules

At present only modified live (MLV) and inactivated PRRSV vaccines are found on the market. Primo-immunization must be done using the live virus. After exposure to the live virus, the specific immune response against the virus can be detected within 2 weeks in most animals and by day 21st after vaccination almost all animals have developed antibodies. However, neutralizing antibodies cannot be detected to significant titers until week 4 post-vaccination. Even then, vaccines usually induce a relatively low neutralizing antibody response (Meier et al., 2003; Diaz et al., 2006, Madapong et al., 2017). In general, it is considered that four weeks is a reasonable period for having efficient immunity after vaccination with an MLV, even some degree of protection exist after 2-3 weeks. Inactivated vaccines are not suitable for primary immunization although if administered repeatedly immunization can be achieved (Zuckermann et al., 2007; Diaz et al., 2013). In general, inactivated vaccines are more adequate to boost humoral responses in previously immunized animals. Vaccination of primed sows before farrowing with an inactivated vaccine, boosts maternally-derived antibodies and increases the duration of colostral-derived immunity in piglets. For the acclimation of naïve gilts, the most common vaccination schedules include no less than two doses of an MLV before the first service with recall vaccination every third or fourth month. Effective duration of immunity has not been consistently demonstrated but some data suggests that the immune response starts to decrease around the fourth month

after vaccination. In some instances a protocol called 6-60, namely vaccination of sows at 6 days of lactation and day 60 of gestation is also used with the aim to protect the sow in the critical late gestation phase, although no substantial benefit of this strategy has been solidly proven compared to blanket vaccination. In piglets, vaccination with an MLV has been proven to reduce viral transmission (reduction of reproduction rate) in a model where several weeks were left between vaccination and exposure. Most common protocols of vaccination of piglets are vaccination at weaning or in the maternities. Only MLV are able to induce some degree of immunity in piglets with maternally-derived antibodies although interference exist (Fablet et al., 2016, Renson et al., 2019).

Rationale use of PRRSV live vaccines

As with any other live vaccine, the use of live PRRSV vaccines requires some safety considerations. Certainly, a live vaccine works because it contains a replicating agent that induces an immune response similar to that of the wild type virus. However, in this context replication implies shedding and potential transmission of the vaccine strain. It is worth to note that replication in the piglet can be high (Martinez-Lobo et al., 2013). Actually, vaccine-derived strains are found wherever PRRS MLV are used (Murtaugh et al., 2010). Transplacental infection with vaccine strains is also possible if sows are vaccinated in late gestation. Although viremic piglets can eventually born, detrimental effects for the gestation are rarely seen. Replication and shedding of MLV are a risk factor for the generation of recombinant strains with field strains. These recombinant strains are increasingly found circulating in the field (Eclercy et al., 2019; Wang et al., 2019). Measures to minimize this undesired circumstance are based in rigorous vaccinations protocols avoiding vaccination of viremic animals. Simultaneous use of different live vaccines in the same premises (for example one for sows and a second one for piglets) should be avoided as well. Epidemiological surveillance for arising of recombinant strains having vaccine parentals is advisable.

Conclusions

PRRSV vaccines are an essential tool for the control of PRRS which benefits surpass the potential drawbacks caused by its use. Future vaccines should induce a broader and more potent immune response having little or no shedding.

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Hindsight 20/20: What have we learned about influenza A viruses in pigs and people since the 2009 H1N1 pandemic?

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Introduction

Influenza A viruses (IAV) are the causative agents of one of the most important viral respiratory diseases in pigs and humans. Human and swine IAV are prone to interspecies transmission, leading to regular incursions from human to pig and vice versa. This bidirectional transmission of IAV has heavily influenced the evolutionary history of IAV in both species. Interspecies transmission of distinct human seasonal lineages, adaptation followed by sustained and intense within-host transmission, virus migration through live pig transport and trade, and rapid evolution represent a considerable challenge for pig health and production. Although only subtypes of H1N1, H1N2, and H3N2 are endemic in swine around the world, considerable genetic and antigenic diversity can be found in the hemagglutinin (HA) and neuraminidase (NA) genes, as well as the remaining 6 genes. The risk of this pattern, regular human seasonal IAV incursion and IAV evolution in swine, to the human population was brought to the forefront during the 2009 H1N1 human pandemic. The complicated global epidemiology of IAV in swine and the implications for public health and influenza pandemic planning are entangled and will be reviewed in the presentation.

Swine IAV was historically characterized as a seasonal respiratory disease, primarily in weaned pigs with waning maternal immunity. Today, clinical disease in the USA peaks during times of the year associated with fluctuations in temperature and decreased ventilation, similar to the human influenza season (Janke 2013). In US pigs, a primary peak is observed in November-December with a secondary spike in March-April (Walia et al. 2019), with a similar trend in Canada (Poljak et al. 2014). However, contemporary influenza illness and diagnosis can be found at any time of the year (Zeller et al. 2018) in nearly all age groups of pigs, even suckling pigs from sows with high titers of influenza specific serum antibodies (Allerson et al. 2013; Corzo et al. 2014).

Human-swine IAV dynamics

The extraordinary genetic and antigenic diversity of H1 and H3 swine IAV is arguably the greatest challenge to controlling infection and to the development of broadly effective vaccines for swine and for human pandemic preparedness. Further, there remains a paucity of contemporary swine IAV sequence and epidemiologic data in most geographic regions. There is sustained transmission of three main lineages of H1 (Figure 1A) and multiple lineages of H3 from human seasonal IAV established across several decades in global pig populations (Figure 1B), with approximately 30 contemporary well-supported monophyletic HA gene clades in swine. Throughout the history of IAV in swine, incursions of human seasonal IAV have had the most dramatic impact on the evolution of IAV in swine. Following the spread of the 2009 H1N1 swine-origin pandemic (H1N1pdm09) in humans, annual introduction of this human seasonal H1N1 virus into pigs has potentiated a decade of reassortment and diversification of HA, NA, and the other 6 genes in endemic swine lineages (Nelson et al. 2015; Gao et al. 2017; Rajao et al. 2017; Rajao et al. 2018). This diversity has important implications for both swine health and control of IAV using vaccines, and is a challenge for pandemic preparedness for the global public health community.

The diversity of swine IAV globally and the realized human-swine interface is incredibly important in the context of zoonotic IAV infections in humans (called “variants” to distinguish from human seasonal IAV). Antigenically-variable and novel swine viruses pose a threat to humans if human population immunity no longer recognizes the resultant swine lineages, as was dramatically demonstrated by the H1N1pdm09. Significant increases in the detection of variant IAV infections in people began with H3N2 swine IAV in the USA in 2012 (Epperson et al. 2013), particularly swine IAV that have reassorted with H1N1pdm09. Variant cases are regularly reported in the USA (Choi et al. 2015; Greenbaum et al. 2015; Duwell et al. 2018; Pulit-Penaloza et al. 2018) and in other countries (Piralla et al. 2015; Resende et al. 2017; Xie et al. 2018; Lu et al. 2019). The risk of variant infection is likely dependent on animal production systems which might differ in the relative degree of human-pig exposure, the type of animal-human interface (e.g., live animal markets, exhibition practices), the ecology of the virus, and other less tangible factors (Karesh et al. 2012). However, many phylogenetic clades of swine IAV currently circulate without evidence of human transmission or variant cases.

Without the ability to accurately predict which of the 30 current clades of swine IAV may cause variant infections or even pandemics, human vaccine preparedness efforts for swine strains are difficult. The global genetic diversity of swine IAV circulating from 2016 to present and of swine IAV in the USA over the past 6 months demonstrated that most swine IAV were significantly different from the current H1 and H3 components of human IAV vaccines (Anderson et al. 2020). Few of the genetic clades globally detected in swine currently contain a WHO pandemic-preparedness Candidate Vaccine Virus (CVV) and those CVVs that are available might not provide protection given observed genetic and antigenic differences in circulating swine viruses. Since human and swine IAV evolution are inherently tangled, a system to regularly and rapidly prioritize and evaluate evolving swine IAV in the context of human risk should be part of a comprehensive pandemic preparedness plan. A more systematic analysis of swine IAV as a risk to the human population is a priority of the OIE and FAO influenza network (OFFLU). Robust surveillance in swine is a critical component in this effort, with priority given to geographic areas with high levels of swine IAV diversity, rapid evolution, production practices that support viral transmission and migration, and specific animal-human interfaces that promote greater contact between pigs and people. Efforts to antigenically characterize swine IAV through an OFFLU report occur biannually at the WHO Vaccine Composition Meeting, where animal influenza activity data are presented concurrently with human seasonal influenza activity data. If the activity of a particular animal IAV clade is high, variant cases are identified, and/or genetic and antigenic diversity of that clade is significantly drifted from previously recommended pre-pandemic CVVs and current human seasonal vaccine strains, a representative strain may be considered for development of a new CVV. The CVVs are shared among the WHO Global Influenza Surveillance and Response Network (GISRS) and with academic, governmental, and industry partners for research or commercial development (Robertson et al. 2011). Prior to the 2009 pandemic, CVVs were exclusively of avian origin, but more recently, several variant viruses have been selected based on confirmed swine-origin variant infections and antigenic divergence from other CVVs and seasonal vaccines. To make this determination, the cross-reactivities of newly detected swine viruses are tested against monovalent ferret antisera raised against CVVs and/or seasonal vaccine strains and, when available, sera from seasonal influenza vaccinated or exposed humans. To further characterize the antigenic relationships between swine and human IAV, comprehensive temporal antigenic characterization of swine and human seasonal vaccine strains has also been undertaken and will be discussed.

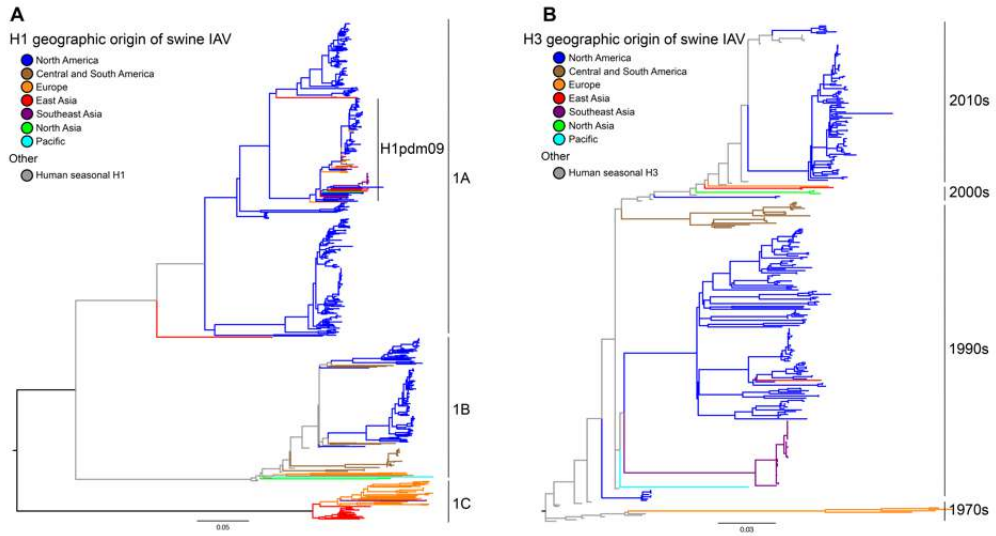

Figure 1. Phylogeny of contemporary swine H1 (A) and H3 (B) influenza A virus hemagglutinin genetic lineages, demonstrating 30 genetically distinct clades that globally co-circulate.

Conclusions

IAV in swine is highly diverse, with sustained transmission in global pig populations of at least 30 genetic clades. The NA and other 6 gene segments also demonstrate a high degree of diversity. Most swine IAV were significantly different at the genetic level from the current H1 and H3 components of human IAV vaccines. Only approximately 1/3 of the 30 distinct genetic clades detected in swine globally currently contain a CVV or human seasonal vaccine, and the degree to which those CVVs provide protection is uncertain given observed genetic and antigenic differences identified in recently circulating swine viruses. Since human and swine IAV evolution are inherently tangled, a system to regularly and rapidly prioritize and evaluate evolving swine IAV in the context of human risk should be part of a comprehensive pandemic preparedness plan. Surveillance in swine must continue to be a priority for animal and public health, with priority given to geographic areas with high levels of swine IAV diversity,

rapid evolution, production practices that support viral transmission and migration, as well as specific animal-human interfaces that promote greater contact between pigs and people.

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Usefulness of ASF diagnostic techniques in the prevention and control of the disease

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Introduction

Although African swine fever (ASF) was first described almost a century ago, controlling the disease has proven to be a challenge, in particular because no vaccine or treatment are available. Spread of ASF can only be prevented by early detection and the application of strict compliance of classical disease control methods, including surveillance, epidemiological investigation, tracing of pigs, stamping out in infected holdings, strict quarantine and biosecurity measures and animal movement control. Epidemiology of ASF is very complex by the existence of different virus circulating which induce different clinical forms, different reservoirs and scenarios depending on the geographical localization, and the on-going spread of the disease through Europe and Asia. Survivor pigs can remain persistently infected for months, which may contribute to virus transmission and thus the spread and maintenance of the disease, thereby complicating attempts to control it (Arias et al., 2018).

Over the last decade, ASF has spread to several European and Asian countries and is now one of the major threats to profitable pig production world-wide (FAO 2020). From the genetic point of view, all the ASFV strains circulating in Europe (except in Sardinia) and in Asia belong to the p72 genotype II and shows high genetic stability with a homology of more than 99.9% (Bao et al., 2019; Forth et al., 2019; Gallardo et al., 2014; Garigliany et al., 2019; Ge et al., 2018; Kim et al., 2020; Malogolovkin et al., 2012; Mazur‐Panasiuk et al., 2019a, b; Le et al., 2019; Rowlands et al., 2008). From the clinical point of view, most of the genotype II isolates of the "Georgia 2007 type" that are currently circulating in Eastern and Central Europe and, now in Asia, are highly virulent and cause very high mortality rates of 91–100% (Pikalo et al., 2019; Zhao et al., 2019). However, experimental and field data provide evidence of the natural evolution of the genotype II ASFVs in Central-Eastern Europe from virulent to attenuated strains able to induce different ASF clinical forms from acute to subclinical infections. These different clinical forms are coexisting in the field, in more or less proportion (Gallardo et al., 2019a, b; Nurmoja et al., 2017; Sargsyan et al., 2018; Zani et al., 2018).

Given the demonstrated clinical evolution of the disease in some affected areas in Europe, mainly in those areas where the ASFV persist within the wild boar population, every country should update the contingency plan and the early warning system in place to prevent the ASFV entry in free areas, its spread, and subsequent maintenance. Any delay in outbreak response and implementation of control measures can result in greater viral contamination of the environment and promote disease spread (Bellini et al., 2016). Highly virulent ASFV isolates are associated to evident clinical forms and should therefore be easier to detect by passive surveillance. However, the demonstrated presence of animals that survive from sub-acute infections or even are subclinical infected, makes the passive surveillance not sufficient for early disease detection in the case of infection with moderately virulent or attenuated ASFV isolates. This is particularly important in high-risk areas. In case of wild life infected animals a combination of passive surveillance of dead wild boar and active surveillance in areas at highest risk should be considered (Arias et al., 2018). The active surveillance will also provide very valuable data on the evolution of the disease and guidance on the assessment of the effectiveness of the control measures. A surveillance system, to be successful, must have adequate laboratory support for a rapid diagnosis, being a key step to design effective control and eradication programs.

Available ASF diagnostic tests

Availability of reliable and accurate diagnostic assays is a prerequisite for an efficient disease control, as any clinical suspicion of ASF in domestic pigs and wild boar has to be verified by laboratory diagnostic methods. On the international level, laboratory methods as well as sampling and shipping guidelines can be found in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Chapter 3.8.1, 2019 edition) and the respective EU Diagnostic Manual (European Commission Decision EC 2003/422/EC).

The starting point for any laboratory investigation of ASF is sample collection. An important consideration is the purpose of the investigation, for example disease diagnosis, disease surveillance, or health certification. Which animals to sample will depend on the objective of the sampling. For example, when investigating an outbreak (passive surveillance), sick and dead animals should be targeted, while the oldest animals should be sampled when checking if animals have been exposed to the disease (active surveillance) (Beltrán-Alcrudo et al 2017). To be effective, proper samples combined with the selection of diagnostic methods, is of fundamental importance in order to make a rapid and

reliable diagnosis. Samples collected from live pigs should include anticoagulated whole blood for the detection of virus or viral nucleic acid and serum for the detection of antibodies, whereas samples collected from dead pigs should comprise tissues from virus detection using. Target organs are spleen, lymph nodes, liver, tonsil, heart, lung, and kidney. Of these, spleen and lymph nodes are the most important as they usually contain the highest amounts of virus. Bone marrow is also useful in incidents involving dead wild animals, as it might be the only tissue that is comparatively well preserved if an animal has been dead for some time. Intra-articular tissues of joints can be examined to check for the presence of low virulent isolates. Tissue fluids are also useful for serological investigations (Gallardo et al., 2015a, 2019b).

In certain situations, sampling can be the bottleneck of swine fever diagnosis, especially in the case of wild boar, but also in remote areas. For this reason, alternative sampling strategies and sample matrices (oral fluids, dried blood-spots on filter papers and swabs) have been tested for ASF (often combined with Classical swine fever sampling) especially for wildlife specimens and under rural conditions. However, most of them are not yet in routine use and need further validation (Blome et al., 2014; Braae et al., 2015; Davies et al., 2017; Grau et al., 2015; Michaud et al., 2007; Mur et al., 2013; Petrov et al., 2014; Randriamparany et al., 2016).

For agent identification, nucleic acid detection tests (real-time PCR, gel-based RT-PCR), virus isolation and haemadsorbing (HAD) assay or antigen detection tests like direct fluorescent antibody test (DIF) on fixed cryosections of organ material and enzyme-linked immunosorbent assays (ELISAs) detecting p72 antigen are available (OIE 2019) (**see table 1**). Antigen ELISA is a rapid method which can be fully automated; however, its sensitivity is rather low (Gallardo et al., 2015a; 2019b; Oura et al., 2013); therefore, it is not to be used for testing individual animals. ASF antigen detection by DIF yields quick results, and is a highly sensitive test for cases of peracute and acute ASF. It is a robust test, but has been largely replaced by PCR and reagents are no longer widely available. It is important to note that in subacute and chronic disease, where antibodies are present, the sensitivity of this method is also limited (40%) and interpretation of test results is difficult and requires well-trained and experienced laboratory staff.

Virus isolation (VI) and identification by HAD tests, a characteristic feature of the ASFV-infected cells, are recommended as a reference confirmatory tests in the event of a primary outbreak or a case of ASF (EC 2003). In theory, all of the ASFVs collected from natural outbreaks can be isolated in susceptible primary leukocyte cultures of swine origin, either from blood or lung (alveolar) monocytes or from macrophages cells. However, growing ASFV isolates is a critical step for diagnosis at the national reference laboratories, since it is more expensive than other techniques, requires both specialized facilities and training, is time consuming and cannot be adapted to high throughput. In addition, attempts to isolate infectious virus from field-derived samples provide irregular results. The reason lies in the poor state of samples received, which affects the virus viability, especially on samples obtained from dead or hunted animals, such as wild boar (Gallardo et al., 2015a, 2019b). Despite these constraints, virus isolation is essential to obtain virus stocks for future molecular and biological characterization studies. The use of established cell lines, such as COS-1, IPAM or wild boar lung cells (WSL), can overcome the difficulty in obtaining the primary cells but they are not always suitable for the ASFV isolation from field samples without a little apparent adaptation (Carrascosa et al., 2011; Gallardo et al., 2013). Therefore, further evaluation studies are required for the potential use of established cell lines in routinely diagnosis.

Among the above listed tests the PCR is by far the most sensitive method for the detection of the agent and should be regarded as the method of choice for first-line laboratory diagnosis. A variety of PCR tests, including both conventional and real time (rPCR), have been developed and validated to detect a wide range of ASF isolates belonging to different known virus genotypes, non-HAD strains, and diverse virulence (Agüero et al, 2003; Fernández-Pinero et al, 2013; King et al, 2003; Tignon et al, 2011; Zsak et al, 2005). All of them have been designed in the VP72-coding region, a highly conserved gene coding the major viral protein, assuring the (potential) detection of any ASFV isolate (Oura et al., 2013; Gallardo et al., 2015a). The OIE rPCRs developed by King et al. (2003; OIE 2019) and the OIE Universal probe library (UPL) rPCR developed by Fernández-Pinero et al., (2013; OIE 2019) are the most widely used for routine diagnosis at the EU´s national reference laboratories (NRLs) level (Nieto R., personal communication 2018). Both methods are able to provide a confident ASF diagnosis, although the UPL-PCR has greater diagnostic sensitivity for detecting survivors and allows earlier detection of the disease even when the typical clinical signs are not yet evident (Fernández-Pinero et al., 2013; Gallardo et al., 2015a). Finally, the number of commercial kits for ASFV genome detection based on published rPCRs, has greatly increased over recent years (table 1). These represent an alternative that can guarantee a certain homogeneity in results, which is important in establishing testing procedures to be adopted by many laboratories. Each of the new ASF-commercial assays must to be evaluated and validated following international guidance to ensure they are specific, sensitive, reproducible, precise, robust and accurate. In summary, the PCR is a basic diagnostic tool for surveillance considering the long-term viremia and high viral load that exhibits in the infected animals suffering acute or subacute clinical courses. It is quick and can be used for individual as well as pooled samples. However, the occurrence of low virulent strains in recent years within the EU has made the diagnosis more problematic as some of these strains show only a short period of viremia (Gallardo et al., 2015c; 2018; 2019a).

Table 1: African swine fever validated ASFV and antibody detection tests.

DETECTION	AVAILABLE TESTS	TYPE, In house/ Commercial	Recommended Use
Virus	Genome detection	PCR (*OIE Taqman probe, *OIE UPL probe or *OIE conventional PCR, and commercial kits[a])	Suspicious Surveillance Individual and Herd testing
	Virus isolation	*VI /Haemadsorption (HAD) test (i.h.)	Confirmation of primary outbreak.
	Antigen detection	*Direct Immuno fluorescence (DIF) (i.h.)	Individual testing (acute forms)
		Antigen ELISA comercial kit INgezim PPA DAS, Double Ab Sandwich	Surveillance Herd testing (acute forms)
	Penside test	Lateral flow device (LFD) commercial kit (INgezim ASF CROM Ag)	Herd testing (acute forms)
Antibody	ELISA	ELISA (OIE, commercial kits[b])	Surveillance Herd testing
	Confirmatory test	*Immunoblot (IB) Test (i.h.)	Confirmatory Herd testing
		*Immunofluorescence Antibody (IIF) test (i.h.)	Confirmatory Herd testing
		*Indirect Immunoperoxidase test (IPT) (i.h.)	Confirmatory Herd testing
	Penside test	LFD commercial kit INgezim PPA CROM	Herd testing

*Included in the OIE Terrestrial Manual for Diagnostic Test and Vaccines, 2019; i.h. (in house methods); a) PCR Commercial Kits currently validated: INgene q PPA, INGENASA. 11.PPA.K.5TX/Q; Tetracore TC-9017-064; Virotype ASFV PCR Kit, INDICAL BIOSCIENCE; LSI VetMAXTM Thermo Fisher Scientific; IDEXX RealPCR ASFV Mix,IDEXX; ID Gene® African Swine Fever Duplex – IDVet; ADIAVET ASFV REAL TIME 100R, BIO-X DIAGNOSTICS. b) ELISA Commercial Kits currently validated; INgezim PPA COMPAC competition-ELISA, INGENASA; ID Screen® ASF Indirect ELISA, IDVET; ID Screen® ASF Competition-ELISA, IDVET; SVANOVIR® ASFV Indirect-ELISA, SVANOVA

Whenever the suspicion is raised that ASFV is circulating in a pig population, serological assays must also be used for the diagnosis of disease. Moreover, serology is applied for surveillance purposes and is a valuable tool for further epidemiological investigations, for example, for determining the time point of agent introduction into a pig herd or into a wild boar population. Anti-ASFV antibodies appear soon after infection and persist for up to several months or even years (Arias and Sánchez-Vizcaino 2002, 2012; Arias et al., 2018; Gallardo et al., 2015a). Additionally, no vaccine is available against ASFV, which means that the presence of anti-ASFV antibodies always indicates infection. Antibody-based surveillance is therefore essential for the detection of surviving animals, to elucidate the epidemiological characteristics of the epidemics, i.e., time since the virus introduction into a farm, and for detecting incursions involving low virulence ASFV isolates (Arias et al., 2018; Gallardo et al., 2015a, b, 2019b; Laddomada et al., 2019). The use of antibody detection assays was also crucial for successful eradication programs in the past (Arias and Sánchez-Vizcaíno 2002, 2012). Current ASFV antibody-based tests approved by the OIE involve the use of an ELISA for antibody screening, backed up by Immunoblotting (IB), Indirect Immunofluorescence (IIF) or the Indirect immunoperoxidase tests (IPT) as confirmatory tests (OIE 2019). The IPT has been proved as the best test for ASF serological diagnosis due its superior sensitivity, but moreover, its performance to test any kind of porcine material such as blood, exudate tissues or body fluids (Gallardo et al., 2015a; 2019b). This is particularly relevant for wild boar surveillance and control programs, where poor sample quality can result in false-positive or false-negative reactions in ELISA test kits, a problem which is in particular observed in wild boar specimen.

The use of the pen-side tests offers a first-line diagnosis that can be useful for rapid application in case of sanitary emergency. The time elapsed between the clinical suspicion and laboratory confirmation used to be relatively long due to the logistics of sending samples to official laboratories. On the other hand, in most cases, regional laboratories do not have the expertise, equipment and/or facilities to diagnose exotic diseases such as ASF. This can be sorted out by the use of pen-side tests for a first front-line diagnosis under field conditions, giving real-time data

on the animal's infection status. Two different lateral flow devices (LFDs) for the detection of antibodies or the viral antigen in blood are commercially available by INGENASA so far, and it is expected several others come in the near future. However, from the published data, the LFDs should not be used alone due to a very limited sensitivity compared to the gold standard methods, overall in the case of the LFD for the detection of antigens (Gallardo et al., 2019b). It is important to point out antibody LFD penside tests are not useful for the detection of acute forms of the disease. They need to be accompanied by virus/antigen detection techniques, due to antibodies cannot be detected before 12-14 days post infection using these tests. The analysis of suspicious samples by both, virus and antibody detection techniques/penside tests will give us a complete picture of what is going on. Test sensitivity needs to be high, whereas specificity is less critical, since any positive result will need to be verified by the competent National reference laboratory. Now, the possible contribution of pen-side tests to the control of notifiable diseases is still discussed controversially and their application may be limited due to national and international regulations. In general, there are a number of commercial diagnostic test kits and reagents on the market and routinely used in the laboratories; however, the quality of the products can vary considerably and in particular, in case of an outbreak scenario, availability of certain test kits on short notice can be problematic.

Taken together, sensitive, specific and robust laboratory diagnostic assays are available; nevertheless, continuous improvement of diagnostic tests in terms of sensitivity, specificity, costs, practicability and robustness is necessary. For the control of the disease, it is important to push the worldwide implementation of already available modern diagnostic techniques in the laboratories involved in ASF diagnosis further ahead. Proficiency laboratory tests, organized by the EU reference laboratory for ASF on a yearly basis, regularly evaluate the status quo of the currently performed diagnostic assays in the EU member states and third countries, thus representing a valuable tool for identification of general gaps in laboratory diagnosis of ASF as well as individual laboratory deviations.

All newly developed diagnostic tests need to be validated appropriately prior to implementation in routine diagnostics. However, while there are general guidelines for validation of diagnostic tests (e.g., OIE), there are no standardized procedures for validation of specific tests, as the respective criteria have not yet been defined. In this context, the development of standardized reference material (e.g., non-infectious molecular standards for nucleic acid detection assays and reference serum standards for ELISA batch testing) could help to increase the comparability of test validation between laboratories. Development of new tests, improvement of existing ones or even maintenance of fully validated kits currently on the market are time- and labour-consuming tasks; therefore, cooperation between laboratories engaged in diagnostics and research with expertise in diagnostic techniques and commercial companies with expertise in licensing and marketing such products is very useful.

The ASF diagnostic interpretation

As in any other disease, there is not a single test being 100% reliable (sensitive and specific). For this reason, final diagnosis should be based on the interpretation of the results derived from the use of a number of validated tests, in combination with the information coming from disease epidemiology, scenario, and the clinical signs. A detailed understanding of the time course of viremia and antibody seroconversion during the ASFV infection is a prerequisite to obtain relevant information about the dynamic of the infection in affected areas and to support control and eradication programs. An appropriate diagnosis therefore should involve the detection and identification of ASFV-specific antigens or DNA and antibodies.

On the basis of experimental data gained at the EURL, upon infection with virulent ASFV strains weak viremia (Cycle threshold Ct >35) can be detectable by real time PCR at on average of 3.75 ± 1.4 days, two days before the onset of the clinical signs. No antibodies are developed. Therefore, a weak PCR positive result on a field blood sample in absence of antibodies could be an indicator of an early phase of the infection (< 1week). Spleen, lymph nodes, liver, and lungs are the sites of secondary viral growth and after 24-30 hours of the primary viremia all tissues contained the virus, reaching the maximum titres around 7-8 days to being infected. The ASFV is therefore easily detected in any kind of porcine sample by real time PCR and even using the antigen detection techniques (DIF or ELISA). A weak antibody response can be detected as early as 7-8 days by IPT in sera and in exudate tissues, mainly spleen and lung, 2-3 days before than with the ELISA (in sera), although with the latter it is rarely detected. The mortality reachs up 92% to 100% within 4 (peracute form) to 12 (acute form) days after the infection.

Since acute forms are predominant at the beginning of outbreaks, the measures taken in free areas bordering infected areas is based on a risk assessment and on enhanced passive surveillance and PCR testing (SANTE/7113/2015 – Rev 11). However, false positive PCR results, although rare, can occur (e.g., due to lab contamination or other factors). It is unlikely that a primary outbreak (or case) of ASF would be made on the basis of a PCR positive result alone. The most conclusive evidence of infection is isolation of ASFV, but there will be situation in which this is not possible since is time-consuming, requires specialized laboratory and staff and, in comparison with the PCR has limited sensitivity, particularly on samples obtained from dead or hunted wild boar, or in weak positive PCR samples (Gallardo

et al., 2015a, 2019b). The occurrence of ASF virus infection can be confirmed if clinical signs or lesions of disease have been detected in the pigs in question and at least two distinct virus or antibody detection tests have given a positive result on samples taken from the same-suspected pig. In wild boar, if virus isolation is not possible, a primary case of ASF can be confirmed when at least two virus or antibody detection tests have given a positive result (EC 2003).

Where ASF becomes endemic, increased numbers of subacute, chronic, and subclinical infections occur and that mortality rates decline over time (Gallardo et al., 2015a, 2015c, 2018, 2019a, b). In clinical terms, subacute ASF develops over a 10-20-day period and the mortality rate ranges from 30 to 70% after 20 days post infection (Arias and Sánchez-Vizcaino, 2012; Beltrán-Alcrudo et al., 2017). Viremia can be detected by real time PCR on average of 8.5 ± 3.6 days and antibodies by ELISA and IPT from 10 days, reaching mean antibody titres of 1:20,000 from the third week. All tissues obtained from animals that succumb within the first month to the infection are positive by PCR and in IPT (Gallardo et al., 2018). The presence of ASF is therefore easily confirmed combined both virus and antibody detection tests, in either blood, sera or tissue samples.

In recovered pigs surviving acute or subacute infections, the viremia, clearly detectable within the first month, decline over the time and only weak PCR results (Ct >35) can be sporadically detected for up to 78 days (Gallardo, et al., 2018). The common feature in these survivors is the presence of high antibody titer in either blood, sera or tissue samples for the entire life of the animal. Nevertheless, the detection of antibodies in a field sample that resulted PCR weak or negative, is not only an indicator of the presence of survivors from acute or subacute infections. Animals infected with non-HAD and low virulence strains seroconvert after the first week of the infection even in absence of clinical signs or viremia. The antibodies are easily detected by IPT and ELISA, reaching antibody levels >1:160,000 after one month which are maintained over the time (Gallardo et al., 2015c, 2019a; Leitao et al., 2001; Sánchez-Cordon et al., 2017; Sánchez-Vizcaíno et al., 2015). The relative low percentage of non- HAD viruses isolated within the EU could come to the fact that these non-HAD viruses are more difficult to isolate than HAD viruses since the viraemia they cause is sporadic and virus has mostly been isolated in small amounts from the organs. Infection of wild boar with these attenuated non-HAD isolates may account for the seropositive animals detected in the field in absence of clinical signs and viremia. These data emphasize the fact that early detection based only on clinical signs and ASFV genome detection is not an efficient approach for the control of ASF in the current epidemiological situation in Europe. It is likely that the European wild boar is getting endemically infected in certain regions within the EU becoming a recurrent source of infection to other wild boar but also, to domestic pigs. Since each animal could be at a different stage of the disease, both virus and antibody detection tests, for confirming transient viremia and the presence of anti-ASFV-specific antibodies, could make sub-clinically ASFV infected wild boar or domestic pigs detectable. A positive test for the presence of the virus indicates that the tested animal was undergoing infection at the time of sampling. On the other hand, a positive ASFV antibody test indicates an ongoing or past infection, where the animals have recovered (and may remain seropositive for life; Tab.2, Fig. 1).

Table 2. Interpretation of the ASF diagnostic results.

ASSAY	RESULT	PROBABLE SCENARIOS
PCR Ab-ELISA IPT	WEAK $_{(Ct>35)}$ NEGATIVE NEGATIVE	Animal has recently infected and it has not yet seroconverted (< 7 days). Clinical signs cannot be evident.
PCR Ab-ELISA IPT	POSITIVE NEGATIVE POSITIVE	Animal has recently infected, develop clinical signs and is initiating the seroconversion (7-10 days)
PCR Ab-ELISA IPT	POSITIVE POSITIVE POSITIVE	a) Infection in course. Animal is still viremic with clinical signs and has already seroconverted (>10 days). b) Reinfection of an animal with preformed antibodies from a previous infection (survivor)
PCR Ab-ELISA IPT	WEAK $_{(Ct>35)}$ or NEGATIVE POSITIVE POSITIVE	a) Past infection. Animal has been recovered from acute or subacute infection and did not present clinical signs. b) Animal infected with attenuated strain (with or w/o clinical signs). c) Reinfection of an animal with preformed antibodies from a previous infection (survivor).

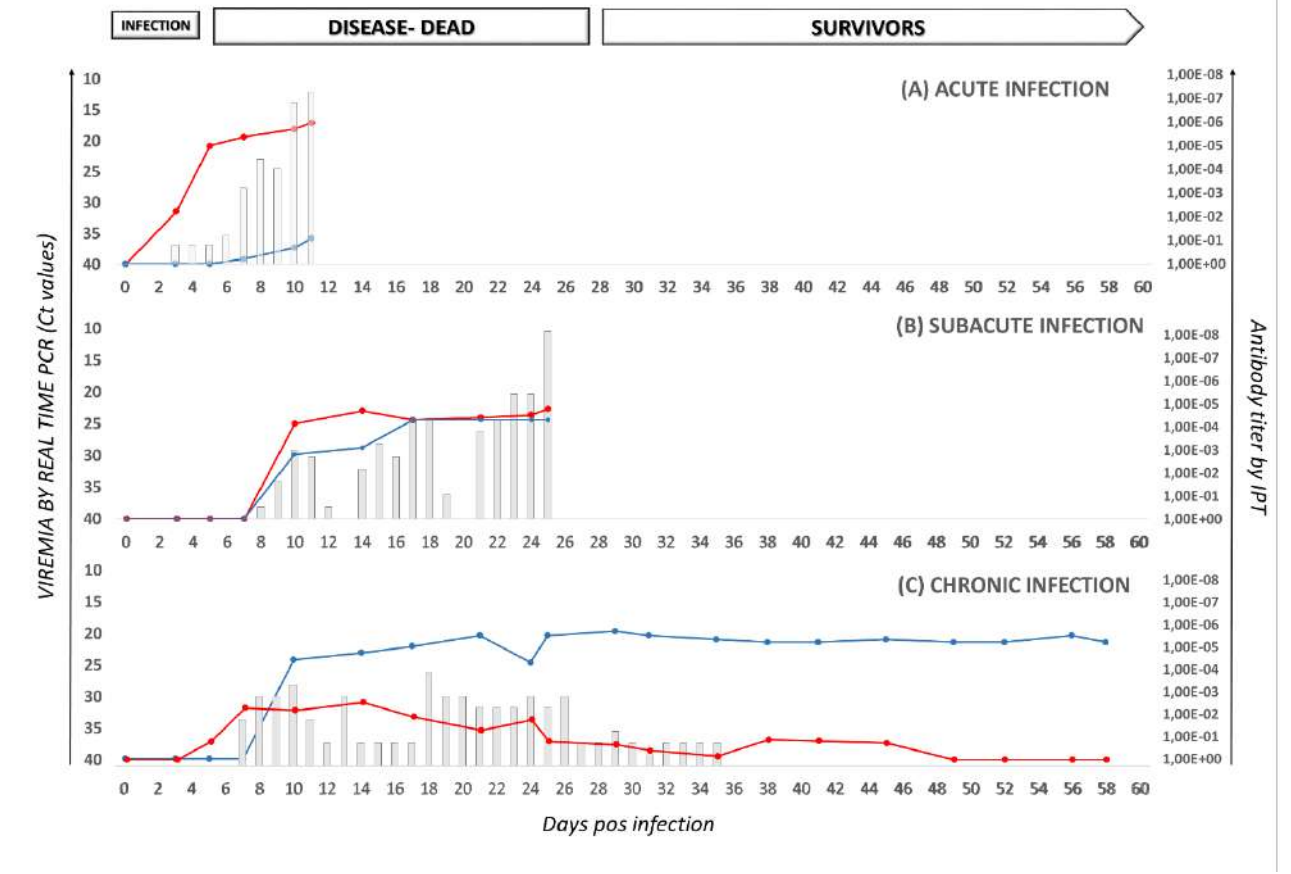

Figure 1. Viremia (measured by real-time PCR) and antibody response (determined by IPT) over time and in relation to the stage of ASF virus infection, as observed in European domestic pigs infected with genotype II ASFV isolates circulating in the EU (2014-2019). Clinical score, expressed in bars, overlapped with viremia and antibody response.

Final remarks

In conclusion, control-eradication programs in areas with a clear endemic tendency should be reviewed and updated and include parallel routine laboratory monitoring, together with the regular clinical inspection. The use of the most fitting diagnostic tools combining both ASF virus and antibody detection will improve the efficacy of disease-control measures, regardless of the nature of the circulating ASFV strains (Arias and Sánchez-Vizcaíno 2002, 2012; Gallardo et al., 2015a, 2019b). An accurate evaluation of the results of the serological and virological tests must be carried out, taking into account all the clinical and epidemiological findings, in the framework of the enquiry to be carried out in case of suspicion or confirmation of ASF.

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Why should we worry about farrowing systems for sows: insights from studies on maternal behavior?

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Maternal investment, sow behavioural needs and criteria for housing design

The reproductive strategy of producing large broods and smaller young, resulting in a higher mortality rate, fits the picture of domestic pigs remarkably well (reviewed by Drake et al. 2008). The average litter size in domestic sows is around 12 to 14 piglets, but litters of above 20 piglets are not uncommon even in gilts, although the number of functional teats is usually between 14 and 16 (e.g. Vasdal & Andersen, 2011; Ocepek et al., 2016). This is due to a high selection pressure for increased number of liveborn piglets. However, a high litter investment (i.e. litter weight at weaning plus weight of stillborn piglets), is associated with a higher weight loss during lactation and a higher prevalence of shoulder lesions, especially in primiparous sows that still need resources for their own growth and development (Ocepek et al., 2016). Thus, breeding for an increased maternal investment at a young age is accompanied with substantial costs for the sows, with potential negative consequences for the residual reproductive value and longevity. There is a strong neonatal competition for teats even when the number of functional teats equals the number of offspring as some piglets may in fact monopolize two teats shortly after birth (Andersen et al., 2011; Ocepek et al., 2017a). During this competition, some piglets will not get access to a teat during milk let-down, will give up fighting after several unsuccessful attempts, and within 2 or 3 days, they will starve to death (e.g. Andersen et al., 2011). Piglet mortality caused by maternal crushing of piglets, many of which has no teat success, and starvation caused by sibling competition, increases with increasing litter size for most sow parities (e.g. Andersen et al., 2011; Ocepec et al., 2017a). A relatively constant number of surviving piglets at the time of weaning, therefore, suggests that 10 to 12 piglets could be close to the upper limit that the domestic sow can take care of without human assistance. However, some pig breeding companies claim that this biological threshold can be stretched even further and are not willing to reduce the weight on number of liveborn piglets in the breeding goal. Other negative consequences of larger litters are that a higher proportion of nursing's is terminated before milk let-down ("unsuccessful" nursing), that nursing interval increases (fewer nursing's per day), resulting in more variation in piglet weight and thus quality of the young at weaning (Ocepek et al., 2017a). Our results suggest that further artificial selection for larger litters in maternal lines is not sustainable because it compromises sow and piglet welfare and fitness. Larger litters are also more labor requiring for the farmer at the time of farrowing.

Because the sows have become larger, heavier and longer, the udder has become larger and the teat pair distance has increased especially in middle and posterior teats (Ocepek et al., 2016). As a result of this, more than 20% of the teats are inaccessible for the new born piglets at the time of birth (Ocepek et al., 2016), either because they are hidden towards the floor or because they are placed too high and out of reach (Vasdal & Andersen, 2011). Furthermore, this problem increases with increasing sow parity. Teat and udder quality traits are therefore extremely important and needs to be addressed with a simple and efficient methodology, for instance by using the tools of digital picture analysis targeting the part of the udder that is most problematic regarding teat pair distance and accessibility for the piglets.

Some behavioural traits important for piglet survival and litter weight, documented both experimentally with pure breeds and on crossbreds with 895 sows from 45 different commercial farms (Ocepek & Andersen, 2017; Ocepek et al., 2017b; Ocepek et al., 2018), are nestbuilding activity, sow-initiated communication with piglets and sow carefulness while being active in the pen. High levels of sow-initiated communication (i.e. sniff, grunt, nudge, push) while resting outside the time of nursing, on the other hand, may indicate restless, nervous behaviour in the sow and thus have negative impact on piglet survival. The scores for sow communication and carefulness were highly correlated, suggesting that we could merge them into one score for future studies and use this score in a breeding program. However, so far, we have not been able to calculate heritability for those important behavioural traits, and neither for the teat pair distance. We hope that some breeding companies would like to collaborate with us to calculate the genetic basis for these traits and then to implement them in the breeding program as these traits appear to have a huge impact on piglet survival and welfare. If the maternal behaviour is optimal this would also lead to less work for the farmer at the time of farrowing.

To provide the sows with free access to relevant nest building material around 12 hours before expected parturition is an excellent routine to improve the maternal motivation in sows. Which material is best suited can be discussed (e.g. Thodberg et al., 1999), but at least when comparing peat with long-stemmed straw, straw resulted in a greater variety of nest building behavioural elements, increased time spent resting and reduced the incidence of oral stereotypies compared to sows that had access to peat or only sawdust (control=minimum amount of sawdust for hygienic purposes is manifested in the Norwegian legislations) in the pen (Rosvold et al., 2018). During farrowing, sows provided with straw or peat as nesting material show a lower frequency of negative communication towards piglets compared to controls (Rosvold et al., 2019). Straw as nest building material also resulted in a higher proportion

of sow-initiated nursing bouts and successful nursing bouts (i.e. with milk let-down) than sows in the peat and control groups. We have also found a shorter farrowing duration compared to sows provided with peat or no specific material, and percentage of stillborn was lowest in the straw group (Rosvold & Andersen, 2019). Another important point to make is that the positive effects of nest-building are also depending on enough space to move around and perform the behavior in a satisfactory way, as for instance crated sows still show bar biting and restless behavior even if they have the same amount of straw as sows in pens (Andersen et al., 2014). This ultimately means that provision of nest building material cannot compensate for the frustration of being confined. Altogether, it thus appears to be a good strategy for stimulating maternal motivation and investment to provide the sows with relevant and enough nesting material with a correct timing prior to parturition. For practical purposes, we recommend that straw is provided from a hay rack with a solid bottom plate and 8-10 cm openings between the rails so that the sows have to work a bit to get it out and to avoid spoiling too much onto the floor and into the slats.

There have been many attempts to develop farrowing pens that meet behavioural needs of sows. The Australian Werribee pen developed as early as in the late 1990s resulted in similar or even lower mortality rates than crates in commercial as well as experimental farms (e.g. Cronin, 1997a; Cronin et al., 2000), and promising results have also been achieved with the FAT2 pens in Switzerland (Weber et al., 2007). An outdoor system with huts could be considered as the most stimulating environment for sows and piglets, but this system is not a realistic alternative in many places due to climatic conditions and because the mortality rate may vary to a large extent. Indicators of piglet survival and the sow traits needed in such a system may be different from an indoor environment (Baxter et al., 2009). In addition to another sow genetic material, this system would require excellent management skills from the producer. More recently developed pens, such as the PigSafe pen, suggest that the nest area should not be too large in order to minimize preweaning mortality. Comparatively, Cronin et al. (1998), suggested that the width of the nest area should be at least 2.2 m, to make it easier for the sow to orientate and nurse. This is also why we chose a pen width of 2.4 in the SowComfort pen decribed below. What is also worth mentioning is that piglets born in the PigSafe pen showed more play behaviour pre-weaning and less aggressive behaviour post weaning than piglets born in a crates (Baxter et al., 2015), suggesting that a more stimulating birth environment may have important developmental effects on piglets.

There are no longer good arguments to confine sows in terms of piglet survival, but there may still be different causes of death in the different systems (Weber et al., 2009; Baxter et al., 2011; Pedersen et al., 2011). In Norway, mortality rate of liveborn piglets in individually loose housed sows have become as low as 12% (InGris National data base, 2019), although number of liveborn piglets are relatively high, with an average of around 14 piglets. Norway banned crates already in 2000, and there are many different farrowing pens on the market that works well and with low mortality. However, as discussed later, mortality of live born piglets and the overall production results, are strongly affected by the farmers management, and more so than the pen design itself. A recent study revealed that crated sows had more locomotion problems and udder lesions, and that the piglets had more skin lesions on their face and carpus than in farrowing pens (Lohmeier et al., 2019). Furthermore, the suckling duration per suckling bout in the same study was longer in the pen system than in crates. From what we know about maternal behaviour of sows, their interactions with piglets and behavioural needs, we do not consider crates or semi-crated systems as satisfactory housing systems for sows during birth and lactation. These systems will thus not be further considered or discussed in the present paper.

It makes little sense to study size of the farrowing pen per se, as this will be the result of including the design elements necessary for sows and her piglets to behave optimal in a pen. As the sows have become larger and longer, the sows need sufficient space and especially wide enough pens for the sow to turn around and orientate within her nest area, both when performing nest-building behaviour (Cronin et al., 1997b; Damm et al., 2003) and when nursing (Cronin et al., 1998). Larger pens with more bedding material, solid walls and not the least with a deeper slatted floor area results in a better dunging pattern and the cleanest pens (Bøe et al., 2019). This is crucially important as the sow needs space to get out of the rest/nest area and into the dunging area with her entire body. Usually nipple drinkers are placed towards neighbouring pens in such a way that she must orientate with her butt part towards the slats and not standing with her front part in the slatted area and the hind part still placed in the solid floor area. If the area is too small and there is no clear distinction between resting and dunging area, the sow will not perform in a way to make the pen clean.

To develop an optimal farrowing environment, we need to understand how the design features of the farrowing environment, management and sow maternal traits interact, and find a combination of these three factors that leads to high piglet survival. The best solution is for the sow to take care of her piglets sometimes with extra work on the part of the farmer around the time of farrowing, even if there is some added labour cost. This requires breeding for good behavioural and physical maternal traits as well as provision of key environmental stimuli fulfilling the needs of the sow to perform certain behaviours and to increase sow motivation to protect her young. Confident sows in a positive emotional state, without painful problems, are likely to become the best mothers.

Key features of a farrowing pen are access to nest-building material before farrowing and the provision of sufficient space for the sow to turn around and orientate within her nest area, both when performing nest-building behaviour (Cronin et al., 1997b; Damm et al., 2003) and when nursing (e.g. Cronin et al., 1998). Separate nesting and dunging areas are needed. To encourage the sow to farrow in the nest area, some level of separation from humans and other pigs is necessary. This can be achieved by enclosing three sides of the nest area with solid walls, and providing a

non-nesting, activity/dunging area with an open view so that the sow can always see who enters the pen (Cronin et al., 1997a), and the neighbouring sows. Another important objective of the nest area design is to increase the preference of sows to farrow in an area that contains specific features promoting piglet survival. For example, sloped, solid walls within the nest area should be provided for the sow to lean against when descending from a standing posture to a resting position (Damm et al., 2006). The provision of floor heating in the nest area helps new-born piglets to dry faster, reduce heat loss shortly after birth and reduce latency to first suckling. This may result in as much as 7% higher survival rate (Malmkvist et al., 2006). Many farrowing pens are constructed with the assumption that newborn piglets are willing to leave their safe, soft, milk-smelling mother's udder to go to a warm creep area. However, piglets under natural or semi-natural conditions would not leave the safety of the nest and their mother's udder during the first days after birth (e.g. Stangel & Jensen, 1991) as staying as close to the sow as possible would increase survival. Even when there is a high-quality creep area formed like a hut with a small entrance, a thick layer of bedding and automatically controlled heaters, piglets still prefer to rest with their mother for the first two days (e.g. Vasdal et al., 2010). Outside the time of nursing, the sow most commonly communicates to attract the piglets and make them come close to her and stay more in the zone where they can get crushed by their mother, but an increased time spent near the sow in this crucial period does not increase mortality (Melisova et al., 2011). Rather the contrary, the best ticket to survival for a piglet by nature is to stay close to its mother for protection, warmth, comfort and to make sure that an important meal is not missed. Sometimes we tend to misunderstand this biology when we talk about danger zone and that the mother is the source of danger for her young. Maybe we should focus more on how we can stimulate the sow for maternal care? Or find out why sows become stressed and maternal care is impaired in a specific environment?

Excample of a farrowing pen designed to meet behavioural needs of the sow - the SowComfort pen

The SowComfort farrowing pen is as the name suggests designed to meet the sow needs and to stimulate good maternal behavior. There are several essential components that make this a comfortable pen for the sow: she has a separate nest area with walls protecting her against the surroundings, equipped with a mattress that is more comfortable than concrete, floor heating and a hay rack from which both hay and straw are provided. Sloped walls offer support when lying down and protection against piglets being crushed. There is a sharp distinction between the closed nest area and the more open activity area that provides the sow with a good overview of the surroundings and allows the farmer to enter the pen outside the nest area that she is motivated to protect. This ensures predictable handling and a good way for the farmer to approach the sow. It is our understanding that "happy" sows that are in a positive emotional state are also the best mothers and the easiest to handle for the farmer. This can be achieved by playing with the biology rather than against it such as when the sows are left no control of her young in the crate or semi-crated systems and when a separate creep area is provided where the sow cannot see her piglets.

The SowComfort farrowing pen comprises two compartments: a "nest area" and an activity/dunging area. The SowComfort pen provides 7.7 m^2 to allow locomotion before farrowing, and space for piglets from birth to 30 kg after the sow has been moved if this is preferred. The "nest area", covered by a 30 mm thick, hollow rubber mat (Calma; www.kraiburg.com), is designed in a way that allows air space above the concrete floor (Figure 2). This enhances the efficiency of floor heating if insulation is placed underneath the rubber mats on the concrete to reduce conductive heat loss. The reasons for choosing a substantial rubber mat in the nest area were to increase resting comfort of the sow and piglets (thereby the name "SowComfort pen"), to minimise the risk of sow shoulder lesions and lameness, and to minimize knee lesions of the piglets. The activity/dunging area contained the sow feeder and drinker as well as a plastic slatted floor. The slot size was 14 mm in diameter.

The SowComfort pen is designed to facilitate the choice that sows show to farrow at the rear of the pen, in the nest area. They probably make this choice to avoid disturbance, such as that by stock-people who approach the pen from the front. The front dunging area is surrounded by fences made of vertical stainless-steel rods to enable the sow to see her surroundings and provide visual and limited physical contact with neighbouring sows. The SowComfort nest area has solid side walls to provide a closed cave-like environment for the sow and piglets, affording the sow a visual barrier for privacy from neighbouring sow(s) whilst in the nest, and hence some sense of isolation from herd mates. The solid walls and the sloping panels were made of fibreglass, which is a long-lasting, hard wearing material that is easy to clean.

As mentioned above, design features of the nest area have been included specifically to promote piglet survival through good maternal behaviour. For example, in addition to the comfortable mat, the SowComfort pen provides a hay/straw rack for nest-building where the sow can pull out as much material as she is motivated to eat and use. A pilot experiment confirmed that 8 cm openings between the metal bars in the grid of the rack were better than 10 cm openings, because this allowed the sow to place her snout between the bars but required her to spend time dragging the straw out. A solid board at the bottom of the hayrack was preferred to avoid waste on the floor. The sows had free access to hay from the hayrack from one week before predicted birth and throughout the entire lactation period, except for a short period of 24 hours before predicted birth when the hay was replaced with straw for nest building. Uncut straw only was used for the nest building period. After birth, this allowed any wet sawdust and straw to be replaced by a thin layer of dry sawdust on top of the mat to ensure dry and hygienically optimal conditions for the neonates. At the

time of birth, the layer of sawdust was 3-4 mm thick. Two other important features of the nest area are sloping panels along two walls and two, independently controlled, heated-floor zones (Figure 2). Sows prefer to lie against sloping panels when descending from standing to lying posture (Figure 2; Damm *et al*., 2006). Hence, with the ability to control temperature in different floor zones, it is possible to influence where the sow lies relative to her litter. Twenty-four hours before expected birth, both heat zones in the floor of the nest area were set at 34 ºC to provide the piglets with heat irrespective of the birth location in the nest area. Also, early findings show that sows prefer temperatures of around 35 °C the time of birth (Phillips *et al*., 2000). Twenty-four hours after birth, the heat zone towards the end wall of the pen was switched off to make sure that the sows still showed a preference to rest on this particular part of the nest area, even if she had reduced her temperature preference as her milk production increased. The under-floor heat zone towards the right short wall of the nest area was maintained at 34 ºC for most of the lactation period, just being reduced to 30 ºC in the last week of lactation, in order to stimulate the piglets to choose this location for resting when not nursing. This wall is too short for most sows to lean against, and thus most sows preferred the area towards the back wall or the centre of the nest. Room temperature was kept at 18-20 ºC during the data collection and artificial light was kept on between 0730 and 1430 hours.

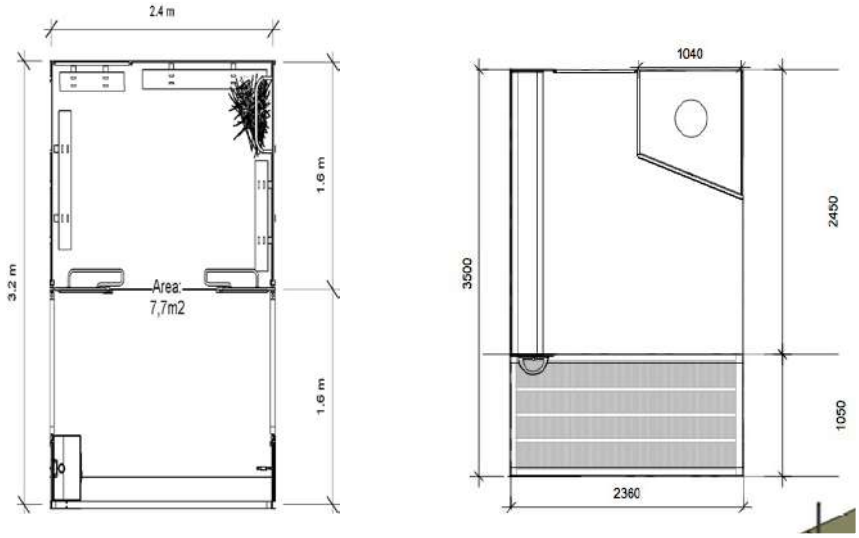

Figure 1. SowComfort pen to the left vs a typical, Norwegian farrowing pen with creep area to the right. The latter had a long through for feeding sows and piglets.

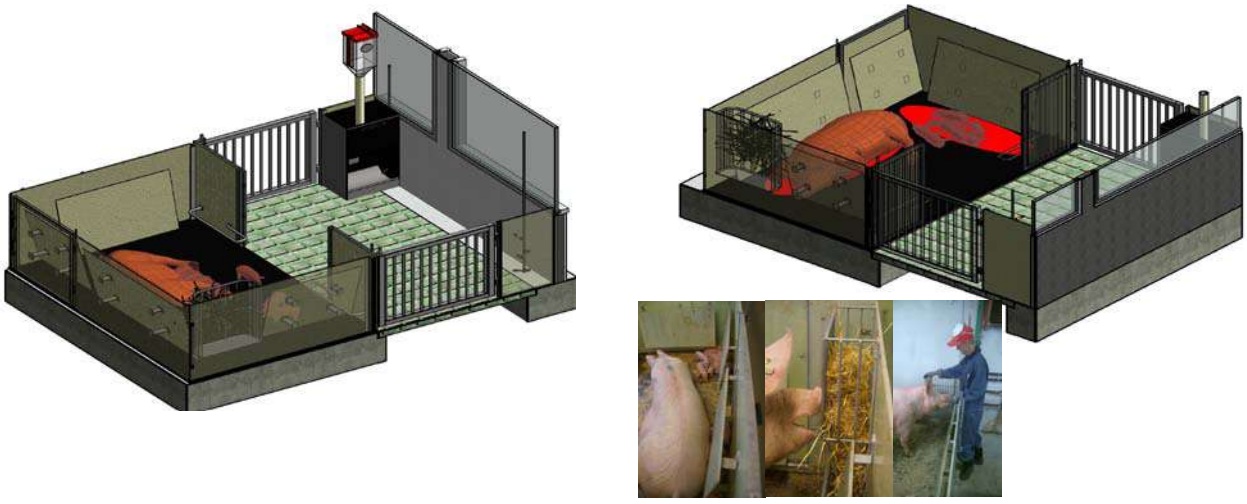

Figure 2. Details of the commercial version of the SowComfort farrowing pen with floor heating areas, rubber mattress on the solid floor, sloped walls in the nest area, hay/straw rack for roughage feeding and nest building material in the nest area. The open design facilitates a good overview for the sow, good contact with neighboring sows and the farmer as the farmer always should enter the pen from the slatted floor area. Drawings were made by Elsbeth Morland in Fjøssystemer A/S.

Figure 3. Commercially improved version of the SowComfort pen with a slightly deeper slatted floor area (10 cm added) and an extra feed dispenser to make an optimal "from birth until 30 kg" pen for the piglets, meaning that the piglets can remain in the birth environment until 30 kg while the sow is removed after weaning at the age of around 5 weeks (legislation demand is no less than 28 days). Drawing was made by May Helen Gryte in Fjøssystemer A/S.

To test the SowComfort pen in a commercial setting, we selected two commercial herds that wanted to build a new farrowing section. From the first commercial herd, we collected data from 162 healthy LY sows of different parities and their litters, of which 61 litters were from three different batches in an old pen system vs. 101 litters were from four different batches in the SowComfort pen (Figure 1). In a second herd, we collected data from 156 healthy LY sows and their litters distributed between three different batches kept in an old pen system. We collected production data from 343 healthy sows with different parities in the new pen, distributed between 7 consecutive batches. The data within each of the two herds were analyzed separately as they differed in management routines, and we used a generalized model in SAS.

Production results showed that % mortality of live born piglets was around 13% in both pen systems in the first herd but causes of mortality in the two pens differed. While the SowComfort pen resulted in lower mortality (1.7 ±0.5%) due to starvation (i.e. no milk in the stomach), more piglets were crushed in this pen compared to the old system (SowComfort pen: 11.8±1.4% vs. old pen system: 9.6± 1.2%). In the same herd, percentage of piglets per litter without knee lesions were significantly higher in the SowComfort pen (28.8±3.1) than in the old pen system (11.0±2.4; $P<0.0001$), indicating that the rubber mattress provides more protection than concrete floor with sawdust.

Table 1. Production results from a commercial herd in the first 7 consecutive batches after installing the SowComfort pen.

SowComfort, 2nd herd	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7
No. of live born per litter	14.5±0.3	14.6±0.3	14.3±0.4	15.7±0.2	13.4±0.3	13.8±0.3	14.2±0.2
Mortality of live born %	15.4±1.6	15.1±1.3	13.0±1.8	**12.3±1.6**	**12.4±1.3**	**12.4±1.8**	**11.7±1.6**
Stillborn %	2.9±0.7	4.8±1.0	4.5±1.2	5.5±1.1	4.6±0.8	6.3±1.2	6.5±1.5
Dead without milk %	3.6±0.7	2.1±0.6	1.0±0.3	1.7±0.6	0.2±0.2	0.7±0.4	1.3±0.5
Overlain %	9.8±1.4	12.1±1.4	10.7±1.7	9.1±1.4	10.7±1.3	9.9±1.8	7.8±1.2
Other causes %	2.7±0.6	0.7±0.3	1.1±0.3	1.7±0.6	1.5±0.5	2.1±0.7	2.4±0.8
No. of weaned piglets	12.2±0.3	12.4±0.2	12.5±0.4	12.3±0.2	13.8±0.4	11.7±0.4	12.2±0.2

In the second herd, where we had the opportunity to collect data from 7 consecutive batches, piglet mortality declined significantly and steadily from 15.4±1.6% in batch 1 to as low as 11.7±1.6 in batch 7 (P<0.0001; Table 1; Figure 4), with the routine that farrowings were usually attended during daytime (80%), but not during the night. Also, sows that had no problems during the farrowing or early nursing, were left as undisturbed as possible. At present, the mortality rate in this particular herd is around 10% with little extra workload at the time of farrowing. Mortality of liveborn piglets was significantly lower in the SowComfort pen (P<0.0001) compared to the old pen system (Figure 4), and primiparous and second parity sows had the lowest mortality (P<0.0001). Mortality due to starvation was rare while crushing was the most common cause of death.

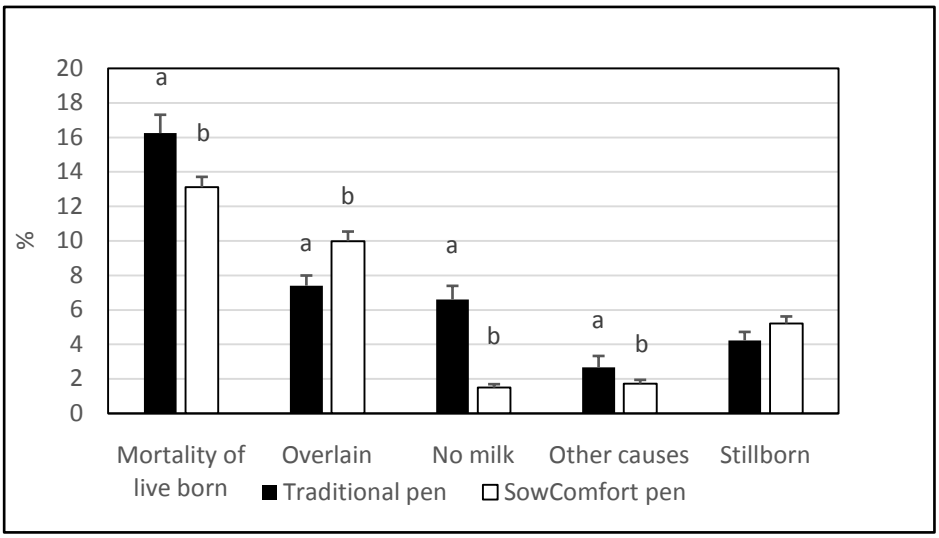

Figure 4. Causes of preweaning mortality in a traditional, individual loose-housed sow pen with a separate piglet creep area compared to the SowComfort pen with a nest area for sows and piglets together and no separate creep area.

Impact of management

To have a functional farrowing pen where the sow is stimulated for maternal care and she has a comfortable place to rest and nurse, is always an important basis for reaching the goal of high productivity without compromising sow and piglet welfare too much. The principle of "working with the biology" rather than battling against it, is the most clear and important advice we can give. Understanding the behavior of the sows and piglets, their activity pattern, their defecation and urination habits, how they communicate etc., are the most important criterias for designing pen systems and for making a list of routines necessary to succeed with this sensitive but fascinating biological system. Loose-housing requires a good human-animal relationship where the sow shows confidence towards the stockperson. To achieve a good relationship with the sows and piglets and positive handling practices, the stock person must show consistent and predictable behavior and attitudes, patience, reinforce positive behavior by petting, friendly talking, and maybe even use treats. What does it really mean to behave in a friendly and respectful way towards the animals while conducting the everyday routines? If you know the answer of that question, there should be less reason to worry about taking the full step from crates to pens where the sow is kept 100% loose. Good, consistent routines around the time of farrowing has major impact on piglet survival until weaning (reviewed by Kirkden et al., 2013). Our recent results from a large survey in 52 Norwegian loose-housed sow herds showed that farmers that attended at least 80% of the farrowings, had frequent positive contact with the sows, dried newborn piglets whenever necessary, and practised split suckling in large litters, had the lowest preweaning mortality (Rosvold et al., 2017). However, split suckling is time consuming especially during batch farrowing when many sows give birth to large litters. When the farrowing pen and creep area is designed in a way that do not protect the piglets from heat loss immediately after birth, piglet survival can be substantially improved by drying the piglets and placing them under a heat lamp (Andersen et al., 2009). This ultimately means that the negative effects of a suboptimal pen can to some extent be compensated by good management practises. Sows that are confident with the stock person and has positive associations with that person is also likely to have fewer negative responses to being assisted during farrowing and will more easily accept that the farmer handles her piglets. This is considered of crucial importance in our commercial farms, and Norsvin (the national breeding company in Norway) is now planning courses in how to observe and interpret behaviours of the pigs in order for the farmer to collaborate more with the animals and have a positive impact in their everyday work at the farm.

The best practice around the time of farrowing can be summed up as follows: 1) make sure that the sow has free access to nest building material around 24 hours before expected parturition (or for the ones that knows the behaviour of their sows: at the time when the sow starts to show restless behaviour from 12 to 6 hours prepartum), 2) the pen should be clean and there should be a generous amount of litter at the time of farrowing, even when using a mattress, 3) the sows that are doing a great job themselves should be left as much in peace as possible, and 4) when present at the time of farrowing, the farmer can assist when the birth process is prolonged and difficult, may prevent crushing during near-crushing accidents, and finally may assist the new born piglets that are lost on the slats or dunging area and needs to be dried and placed at the udder or close to a heat source. 5) Cross fostering of larger piglets is a good routine if some piglets do not get access to a teat (e.g. reviewed by Kirkden et al., 2013), but should not be practised if the sow can feed her entire litter. Artificial rearing is not a good alternative to being reared and nursed by a sow as it impairs behaviour (i.e. more belly nosing, oral manipulation, less play and exploration, lower emotional status), welfare and growth of the piglets (Schmitt et al., 2019).

Conclusions

To answer the question "Why should we worry about farrowing systems for sows?", our claim is that we both have enough scientific knowledge and practical experience in some countries that have had loose housing for many decades, that we should not have fear towards letting the sows loose and choose systems that prioritize good animal welfare. It is more a question of the quickest route to share existing knowledge so that it can benefit the animals and stockpersons at the present stage and not far in the future. To satisfy behavioural needs during confinement is hardly possible, and crates or semi-crated systems should thus be banned altogether because of this, especially when we know that similar production results can be achieved without compromising welfare so much in loose-housed sow pens. While a good pen design forms an important basis for producing high quality litters, we still should keep in mind that it is the sow maternal traits and the management practices that has the largest impact on sow productivity and piglet survival in loose-housed sow herds. This is also why we always should keep track of side-effects of selection and make sure that breeding programs include traits that increases robustness and longevity, and that animal keepers and stock persons have enough competence about welfare as well as productivity.

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African swine fever (ASF) diagnosis, an essential tool in the epidemiological investigation

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Introduction

African swine fever (ASF) is one of the most complex infectious swine diseases. Its notification to the World Organization for Animal Health (OIE) is mandatory due to the high mortality it causes, its efficient transmission rate and the great sanitary and socioeconomic impact that it produces on international trade of pigs and pork products. The African swine fever virus (ASFV) is a large, enveloped double-stranded DNA virus, which is the only member of the Asfarviridae family (Dixon et al., 2005).

Endemic in more than 20 sub-Saharan African countries (Mulumba-Mfumu et al., 2019) and in the island of Sardinia (Italy) since the last century (Cappai et al., 2018; Jurado et al., 2018a; Laddomada et al., 2019), ASF arrived at a Black Sea harbor in Georgia in 2007 (Rowlands et al., 2008), from where the disease spread quickly to other neighboring countries, reaching the European Union (EU) in 2014. The first cases of infected wild boar were reported in January and February 2014 from Lithuania and Poland, respectively, followed by Latvia in June and Estonia in September (Gallardo et al., 2014). In 2017 ASF spread to Romania and Czech Republic, although in the latter was declared as resolved in January 2019. The latest countries affected have been Bulgaria, Hungary and Belgium in 2018, and Serbia and Slovakia in summer 2019. In the EU scenario, the wild boar has been the most severely affected host, being responsible of more than 85% of the reported cases in all countries except in Romania (Cwynar et al., 2019; EFSA 2018a,b; EC 2019; Iglesias et al., 2017; Jurado et al., 2018b).

In August 2018, ASFV demonstrated its huge capacity for transboundary and transcontinental spread jumping to China, several hundreds of kilometers away from previously known infected regions. There, it rapidly spread with 165 ASF outbreaks confirmed in 35 provinces and the culling of more than 1 million pigs by 6 February 2020 (FAO 2020). The continuous spread of ASF to other Asian countries, with confirmed detections in Viet Nam, Mongolia, Cambodia, Democratic People's Republic of Korea, Myanmar, The Philippines, Republic of Korea, Timor-Leste, and Indonesia, makes controlling the spread even harder.

Although ASF was first described almost a century ago, controlling the disease has proven to be a challenge, in particular because no vaccine or treatment are available. Spread of ASF can only be prevented by early detection and the application of strict compliance of classical disease control methods, including surveillance, epidemiological investigation, tracing of pigs, stamping out in infected holdings, strict quarantine and biosecurity measures and animal movement control. Surveillance, to be successful, must have adequate laboratory support for a rapid diagnosis, which in combination with the information coming from disease epidemiology, scenario, and the clinical signs, will allow the early detection of the disease and therefore reduce/prevent ASFV spreading (EC 2013).

ASFV genetic diversity and clinical presentations in affected areas of Central-Eastern Europe and Asia

The molecular phylogeny of the virus is firstly investigated by sequencing the 3'end of the VP72 coding gene, which differentiates up to 24 distinct genotypes (Bastos et al., 2003; Quembo et al., 2018). All the ASFV strains circulating in Europe (except in Sardinia) and in Asia belong to the p72 genotype II (fig. 1) (Gallardo et al., 2014; Garigliany et al., 2019; Ge et al., 2018; Kim et al., 2020; Malogolovkin et al., 2012; Le et al., 2019; Rowlands et al., 2008). Despite different variants have been identified within genotype II strains throughout the analysis of small genome regions (Fraczyk et al., 2016a; Gallardo et al., 2014; Mazur-Panasiuk and Woźniakowski, 2019a; Nieto et al. 2016), the full genome sequence of 17 European genotype II viruses shows a low mutation rate and high genetic stability with a homology of more than 99.9% (Bao et al., 2019; Cano-Gómez et al., 2018; Forth et al., 2019; Mazur‑Panasiuk et al., 2019b). These results hinder the definition of reliable genetic markers associated to virulence. Therefore, the current approach to identify changes in virulence and pathogenesis mechanisms is still based on classical experimental infections and field observations.

Figure 1. Distribution of the ASFV genotypes.

Clinical signs associated with ASFV infection are highly variable depending on various factors: virus virulence, swine breed affected, route of exposure, infectious dose, and endemicity status in the area. According to their virulence, ASFVs are classified as highly, moderately or low virulent (Blome, et al., 2013; Beltrán-Alcrudo, et al., 2017; Sánchez-Vizcaino et al., 2015). Highly virulent strains are usually responsible for the peracute and acute forms that give rise to high mortality rates that may reach 100% within 4–9 days post-infection. In peracute ASF, affected animals can die suddenly 1–4 days after the onset of clinical signs with no evident lesions in organs. Pigs showing the acute forms of the disease display mainly a febrile syndrome with erythema and cyanosis of the skin. Internal lesions are mainly characterized by hyperaemic splenomegaly and haemorrhages in organs, particularly in the visceral lymph nodes, with fluids in body cavities and fibrin strands on organ surfaces. The distribution and frequency of these lesions are variable and most are seen in other swine diseases such as classical swine fever (CSF). Moderately virulent viruses lead to the appearance of acute, subacute and chronic forms. Pigs with subacute infection may have persistent or fluctuating temperature responses for up to 20 days, during which time some pigs stay in good condition, while others display the symptoms described above for the acute process form (but less severely) with mortality rates in the range 30–70%, usually after 20 dpi. In the chronic form of ASF, clinical signs and lesions are not specific and may persist for several months, giving rise to a range of illnesses, with symptoms such as skin ulcers and arthritis, stunted delayed growth, emaciation, lameness, pneumonia, and abortion, but with low mortality rates (Moulton and Coggins, 1968; Mebus and Dardiri 1980; Leitao, et al., 2001; Sánchez-Vizcaíno, et al., 2015).

From the published data, most of the genotype II isolates of the "Georgia 2007 type" that are currently circulating in Eastern and Central Europe and, now in Asia, are highly virulent and cause very high mortality rates of 91–100%. After intramuscular inoculation, the animals, regardless of the host, became infected after an average of 4.4±1.2 days and did not survive for more than 11 days (Gallardo et al., 2017, 2018a, 2019b; Pikalo et al., 2019; Zhao et al., 2019). Both intranasal and oronasal routes are similarly lethal although the nasal route resulted in higher ASF incidence than the oral route when using a lower infectious dose (Guinat et al., 2016). Once infected, the animals developed acute clinical signs between 3.5 to 14 (average) days with the death of 91 up to 100% of the infected animals between 7 to 21 days after the first case (Gabriel et al., 2011; Nurmoja et al., 2016; Olensen et al., 2017; Pietschmann et al., 2015; Vlasova et al., 2015). A similar picture has been observed in pigs exposed to the virus by direct contact with infected animals. The exposed animals developed similar acute clinical signs, which resulted in death between 11 to 25 days post exposure (Blome et al., 2013; Guinat et al., 2014; Gallardo et al., 2017; 2019b; Olesen et al., 2017).

From these experimental data, doubts remained about a potential reduction in the virulence of genotype II ASFV strains circulating in Europe and Asia, and the possibility that domestic pigs or wild boar may develop chronic infections and thus may recover and become carriers. However, the early identification of ASF outbreaks/cases in certain areas of Europe, as the Baltic countries and Poland were the disease has become endemic, has been hampered by the inherent difficulties to recognize the initial signs of infection (Gallardo et al., 2015a; 2018; Nurmoja et al., 2017). It is important to point out that, when introduced into a region or a domestic pig population, ASF is typically associated with high mortality rates and a rapid spread of outbreaks (Sanchez-Vizcaino et al., 2015; FAO 2020). However, even in acute infections, a 2-10% of the infected animals can recover. These survivors may establish a persistent infection in

some tissues and, under certain natural or induced conditions (transport, underfeeding, immunosuppression, etc.) may reactivate the virus, thereby facilitating its transmission. Furthermore, these animals are protected to a secondary ASFV infection, remaining sub-clinically infected, acting as a potential source of infection for the environment and for healthy animals as they could show low levels of viremia (wild boar and domestic pigs). This explain the natural evolution of the ASFVs including the emergence of less virulent forms over time, as occurred in different geographic regions where ASF has been present for a long time (Africa, Iberian Peninsula and Sardinia) (Arias and Sanchez-Vizcaino 2002, 2012; Arias et al., 2018; Gallardo et al., 2015b, 2015c, 2018; 2019b).

Data obtained from the field in areas where the genotype II strains are circulating suggest the evolution of the ASFVs towards less virulence forms. Field epidemiological investigations conducted in Armenia and Estonia described the presence of atypical clinical forms of ASF coexisting with acute typical forms, suggesting the co-circulation of strains of different virulence in the countries (Nurmoja et al., 2017; Sargsyan et al., 2018; Zani et al., 2018). The work developed by Gallardo et al., (2018) with different EU ASFV genotype II strains confirmed the presence of virus of moderate virulence circulating among the wild boar population in Estonia in 2015 and in 2016 able to induce variable clinical signs in the infected domestic pigs ranging from acute, subacute to chronic ASF. Finally, two non-haemadsorbing (non-HAD) genotype II ASFVs were isolated from hunted wild boar in Latvia in February and November 2017 (Gallardo et al., 2019). Domestic pigs infected with these strains developed a non-specific or subclinical form of the disease and were protected from a re-infection with an HAD virulent Latvia strain. Non-HAD virus isolates were previously isolated in the past from regions where ASFV has been maintained for a long time. During the extended epizooty of the last century in the Iberian Peninsula, non-pathogenic and non-HAD viruses were collected in Portugal from pigs and ticks in field areas where most of the herds w ith seropositive pigs were detected (Boinas et al., 2004). Non-HAD strains were also reported in Spain, with a total of 206 non-HAD isolates obtained in the period between 1965 and the first semester of 1974 (Boinas et al., 200 4). Non-HAD isolates have been isolated also in Africa (Gonzague et al., 2001; Pini & Wagenaar, 1974; Thomson et al., 1979).

These data provide evidence of the natural evolution of the genotype II ASFVs in Central-Eastern Europe, from virulent to attenuated strains, able to induce different ASF clinical forms from acute to subclinical infections, which are coexisting in the field, in more or less proportion, depending of the affected region (tab. 1).

Table 1. Biological characterization of African swine fever virus (ASFV) through in vivo studies done at the EURL in domestic pigs with ASFV genotype II strains isolated from wild boar (WB) or domestic pig (DP) within the EU countries.

Asfv Strain		**N°/ Type Of Animals**		**Incubation Period***		**Survival**		**Clinical Form**
		INOC.	*DIRECT CONTACT (RATIO)*	*INOC.*	*DIRECT*	*INOC.*	*DIRECT*	
LT14/1490 (Lithuania 2014)	**HAD**	8 DP	10 DP (1:1.25)	4.5 ± 1	13± 1	0% [8±1]	9% [18± 2.7]	VIR
Pol16/DP-OUT21 (Poland 2016)	**HAD**	2 DP	4 DP (2:1)	5 ± 1.6	9.5±1.9	0% [8±0]	0% [16± 2.4]	VIR
Lv17/WB/Zieme1 (Latvia 2017)	**HAD**	1DP	1 DP (1:1)	2	3	0% [12]	0% [16]	VIR
Bel18/WB/Lux1 (Belgium 2018)	**HAD**	1DP	2 DP (2:1)	5	9	0% [7]	0% [12±1.5]	VIR
Es15/WB/Valga6 (Estonia 2015)	**HAD**	2 DP	4 DP (2:1)	8	10 ± 2.4	0% [17.5±4 .7]	50% [24± 1]	MOD. VIR.
Es15/WB/Tartu14 (Estonia 2015)	**HAD**	2 DP	4 DP (2:1)	4.5 ± 1.3	10 ± 2.4	0% [9±1.6]	50% [25± 6.8]	MOD. VIR.
Es16/WB-Viru8 (Estonia 2016)	**HAD**	2 DP	4 DP (2:1)	5 ± 1.6	9.5 ± 1.9	0% [10±0]	50% [17± 1.8]	MOD. VIR.
Lv17/WB/Rie1 (Latvia 2017)	**NO HAD**	2 DP	4 DP (2:1)	8	11,5	50% [45]	75% [25]	ATT.
Lv17/WB/Rie14/Tukuma5 (Latvia 2017)	**NO HAD**	2 DP	3 DP (1.5:1)	6.5 ± 0.5	13.5± 0.5	50% [9.5]	66.6% [35]	ATT.

*the time from infection to onset of clinical signs; HAD (haemadsorbing); VIR= virulent; MOD.VIR = moderate virulent; ATT= attenuated.

Available ASF diagnostic tests

Given the demonstrated clinical evolution of the disease in some affected areas in Europe, mainly where the ASFV persists within the wild boar population, every country should update the contingency plan and the early warning system in place to prevent the ASFV entry in free areas, its spread, and subsequent maintenance. Any delay in outbreak response and implementation of control measures can result in greater viral contamination of the environment and disease spread (Bellini et al., 2016). Highly virulent ASFV isolates are associated with evident clinical forms and should therefore be easier to detect by passive surveillance. However, the demonstrated presence of animals that survive from sub-acute infections or even are subclinically infected, makes the passive surveillance not sufficient for early disease detection in the case of infection with moderately virulent or attenuated ASFV isolates. This is particularly important in high-risk areas. In case of wildlife infected animals, a combination of passive surveillance of dead wild boar and active surveillance in areas at highest risk should be considered (Arias et al., 2018). The active surveillance will also provide very valuable data on the evolution of the disease and guidance on the assessment of the effectiveness of the control measures. A surveillance system, to be successful, must have adequate laboratory support for a rapid diagnosis, being a key step to design effective control and eradication programs.

On the international level, laboratory methods as well as sampling and shipping guidelines can be found in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Chapter 3.8.1, 2019 edition) and the respective EU Diagnostic Manual (European Commission Decision EC 2003/422/EC). A wide spectrum of accurate ASF diagnostic tests is available, most of them successfully employed in surveillance, control, and eradication programs (see Tab. 2).

The OIE-recommended tests for virus detection include both real-time and conventional PCR assays (Agüero et al., 2003; King et al., 2003; Fernández-Pinero et al., 2013), virus isolation and direct immunofluorescence tests (DIF) (OIE 2019). Virus isolation (VI) and identification by HAD tests, a characteristic feature of the ASFV-infected cells, are recommended as reference confirmatory tests in the event of a primary outbreak or a case of ASF (EC 2003). However, it is not likely to be the most fruitful approach for an effective ASF diagnosis since it is more expensive than other techniques, requires both specialized facilities and training, is time consuming and cannot be adapted to high throughput. In addition, attempts to isolate infectious virus from field-derived samples provide irregular results. The reason lies in the poor state of samples received, which affects the virus viability, especially on samples obtained from dead or hunted animals, such as wild boar (Gallardo et al., 2015a, 2019b). Other assays such as antigen detection ELISA, which allows for large-scale testing of samples, are also available but exhibiting lower diagnostic sensitivity than PCR tests. The use of DIF or antigen ELISA is only recommended as a herd assay and should be combined with some other virological and/or serological tests due to the lack of sensitivity in case of seropositive animals. In conclusion, the real-time PCR is considered the 'gold standard' virus detection test for early detection of the disease and for surveillance. This is due to its superior sensitivity, specificity, robustness and high-throughput application to detect the ASFV genome in any kind of clinical samples from domestic pigs, wild boar and ticks (Gallardo et al., 2015a, 2019; Oura et al., 2013). The use of the real-time PCR as the preferred virological method for routine diagnosis and the need of high-throughput application, added to its worldwide demand, has led to the emergence of a considerable number of commercial kits in recent years. The diagnostic performance of PCR kits should be ensured before being implemented in the lab (table 2).

Serological assays are the most commonly used diagnostic tests due to their simplicity, relatively low cost and need for slight specialized devices or facilities. For ASF diagnosis, the antibody detection is particularly relevant given that no vaccine is available, which means that the presence of anti-ASFV antibodies always indicates infection. Furthermore, anti-ASFV antibodies appear soon after infection and persist for up to several months or even years (Arias and Sánchez-Vizcaino 2002, 2012). Antibody-based surveillance is therefore essential for the detection of surviving animals, to elucidate the epidemiological characteristics of the epidemics, i.e., time since the virus introduction into a farm, and for detecting incursions involving low virulence ASFV isolates (Arias et al., 2018; Gallardo et al., 2015a, b, 2019b; Laddomada et al., 2019). The use of antibody detection assays was also crucial for successful eradication programs in the past (Arias and Sánchez-Vizcaíno 2002, 2012). Current ASFV antibody-based tests approved by the OIE involve the use of an ELISA for antibody screening, backed up by Immunoblotting (IB), Indirect Immunofluorescence (IIF) or the Indirect immunoperoxidase tests (IPT) as confirmatory methods (OIE 2019). Furthermore, the IPT has been proved as the best test for ASF serological diagnosis due its superior sensitivity, but moreover, its performance to test any kind of porcine material such as blood, exudate tissues or body fluids (Gallardo et al., 2015a). This is particularly relevant for wild boar surveillance and control programs.

Finally, the use of pen-side tests offers a first-line diagnosis that can be useful for rapid application in case of sanitary emergency. The time elapsed between the clinical suspicion and laboratory confirmation used to be relatively long due to the logistics of sending samples to official laboratories. On the other hand, in many countries, regional laboratories do not have the expertise, equipment and/or facilities to diagnose exotic diseases such as ASF. This can be sorted out by the use of pen-side tests for a first front-line diagnosis under field conditions, giving real-time data on the animal's infection status. Two different lateral flow devices (LFDs) for the detection of antibodies or the viral antigen in

blood are commercially available by INGENASA so far, and it is expected several others come in the near future. However, from the published data, the LFDs should not be used alone due to a very limited sensitivity compared to the gold standard methods, overall in the case of the LFD for the detection of antigens (Sastre et al., 2016; Gallardo et al., 2019). It is important to point out that antibody LFD penside tests are not useful for the detection of acute forms of the disease since cannot be detected before 12-14 days post-infection. Then, these tests need to be accompanied by virus/antigen detection techniques. The analysis of suspicious samples by both virus and antibody detection techniques/penside tests will give us an initial picture of what is going on. Test sensitivity needs to be high, whereas specificity is less critical, since any positive result will need to be verified by the competent National reference laboratory. The use of pen-side tests for on-farm or field screening requires to be restricted to official veterinarians or regional laboratories with limited resources and should be used taking into consideration a specific circumstances.

Table 2. African swine fever validated diagnostic tests.

Detection	Available Tests	TYPE, In house/ Commercial	Recommended Use
Virus	Genome detection	PCR (*OIE Taqman probe, *OIE UPL probe or *OIE conventional PCR, and commercial kits[a])	Suspicious Surveillance Individual and Herd testing
	Virus isolation	*VI /Haemadsorption (HAD) test (i.h.)	Confirmation of primary outbreak.
	Antigen detection	*Direct Immuno fluorescence (DIF) (i.h.)	Individual testing (acute forms)
		Antigen ELISA comercial kit INgezim PPA DAS, Double Ab Sandwich	Surveillance Herd testing (acute forms)
	Penside test	Lateral flow device (LFD) commercial kit (INgezim ASF CROM Ag)	Herd testing (acute forms)
Antibody	ELISA	ELISA (OIE, commercial kits[b])	Surveillance Herd testing
	Confirmatory test	*Immunoblot (IB) Test (i.h.)	Confirmatory Herd testing
		*Immunofluorescence Antibody (IIF) test (i.h.)	Confirmatory Herd testing
		*Indirect Immunoperoxidase test (IPT) (i.h.)	Confirmatory Herd testing
	Penside test	LFD commercial kit INgezim PPA CROM	Herd testing

*Included in the OIE Terrestrial Manual for Diagnostic Test and Vaccines, 2019; i.h. (in house methods);
a) PCR Commercial Kits currently validated: INgene q PPA, INGENASA. 11.PPA.K.5TX/Q; Tetracore TC-9017-064; Virotype ASFV PCR Kit, INDICAL BIOSCIENCE; LSI VetMAXTM Thermo Fisher Scientific; IDEXX RealPCR ASFV Mix,IDEXX; ID Gene® African Swine Fever Duplex – IDVet; ADIAVET ASFV REAL TIME 100R, BIO-X DIAGNOSTICS.
b) ELISA Commercial Kits currently validated; INgezim PPA COMPAC competition-ELISA, INGENASA; ID Screen® ASF Indirect ELISA, IDVET; ID Screen® ASF Competition-ELISA, IDVET; SVANOVIR® ASFV Indirect-ELISA, SVANOVA

The starting point for any laboratory investigation of ASF is sample collection. An important consideration is the purpose of the investigation, for example disease diagnosis, disease surveillance, or health certification. Which animals to sample will depend on the objective of the sampling. For example, when investigating an outbreak (passive surveillance), sick and dead animals should be targeted, while the oldest animals should be sampled when checking if animals have been exposed to the disease (active surveillance) (Beltrán-Alcrudo et al., 2017). To be effective, proper samples combined with the selection of diagnostic methods, is of fundamental importance in order to make a rapid and reliable diagnosis. Nevertheless, in certain situations, sampling can be the bottleneck of swine fever diagnosis, especially in the case of wild boar, but also in remote areas. For this reason, alternative sampling strategies and sample matrices (oral fluids, dried blood-spots on filter papers and swabs) have been tested for ASF (often combined with Classical swine fever sampling) especially for wildlife specimens and under rural conditions. However, most of them are not yet in routine use and need further validation (Blome et al., 2014; Braae et al., 2015; Davies et al., 2017; Grau et al., 2015; Michaud et al., 2007; Mur et al., 2013; Petrov et al., 2014; Randriamparany et al., 2016).

The ASF diagnostic interpretation

As in any other disease, there is not a single test being 100% reliable (sensitive and specific). For this reason, final diagnosis should be based on the interpretation of the results derived from the use of a number of validated tests, in combination with the information coming from disease epidemiology, scenario, and the clinical signs. A detailed understanding of the time course of viremia and antibody seroconversion during the ASFV infection is a prerequisite to obtain relevant information about the dynamic of the infection in affected areas and to support control and eradication programs. An appropriate diagnosis therefore should involve the detection and identification of ASFV-specific antigens or DNA and antibodies.

On the basis of experimental data gained at the EURL, upon infection with virulent ASFV strains, an starting weak viremia (Cycle threshold Ct >35) can be detectable by real-time PCR at on average of 3.75 ± 1.4 days, two days before the onset of the clinical signs. No antibodies are developed at this early time. Therefore, a weak PCR positive result on a field blood sample in absence of antibodies could be an indicator of an early phase of the infection (< 1week). Spleen, lymph nodes, liver, and lungs are the sites of secondary viral growth and, after 24-30 hours of the primary viremia, all tissues contain the virus, reaching the maximum titres around 7-8 days after being infected. The ASFV is therefore easily detected in any kind of porcine sample by real-time PCR and even using the antigen detection techniques (DIF or ELISA). A weak antibody response can be detected as early as 7-8 days by IPT in sera and in tissues exudate, mainly spleen and lung, 2-3 days before being rarely detectable by ELISA. In this scenario, the mortality can reach up 92% to 100% within 4 (peracute form) to 12 (acute form) days after the infection.

Since acute forms are predominant at the beginning of outbreaks, the measures taken in free areas bordering infected areas are based on a risk assessment and on enhanced passive surveillance and PCR testing (SANTE/7113/2015 – Rev 11). However, false positive PCR results, although rare, can occur (e.g., due to lab contamination or other factors). It is unlikely that a primary outbreak (or case) of ASF will occur only based on a PCR positive result. The most conclusive evidence of infection is the isolation of ASFV, but several situations limit or even prevent its application. Virus isolation is time-consuming, requires specialized laboratory and staff and, in comparison with the PCR, has limited sensitivity, particularly on samples obtained from dead or hunted wild boar, or in weak positive PCR samples (Gallardo et al., 2015, 2019). The occurrence of ASFV infection can be confirmed if clinical signs or lesions of disease have been detected in the pigs in question and at least two distinct virus or antibody detection tests have given a positive result on samples taken from the same-suspected pig. In wild boar, if virus isolation is not possible, a primary case of ASF can be confirmed when at least two virus or antibody detection tests have given a positive result (EC 2003).

Where ASF becomes endemic, increased numbers of subacute, chronic, and subclinical infections occur and mortality rates decline over time. In clinical terms, subacute ASF develops over a 10-20-day period and the mortality rate ranges from 30 to 70% after 20 days post infection (Arias and Sánchez-Vizcaino, 2012; Beltrán-Alcrudo et al., 2017). Viremia can be detected by real-time PCR on average of 8.5 ± 3.6 days and antibodies by ELISA and IPT from 10 days, reaching mean antibody titres of 1:20,000 from the third week. All tissues obtained from animals that succumb within the first month to the infection are positive by PCR and in IPT (Gallardo et al., 2018a, b). The presence of ASF is therefore easily confirmed combining both virus and antibody detection tests, in either blood, sera or tissue samples.

In recovered pigs surviving acute or subacute infections, the viremia, clearly detectable within the first month, declines over the time and only weak PCR results (Ct >35) can be sporadically detected for up to 78 days (Gallardo, et al., 2018). The common feature in these survivors is the presence of high antibody titer in either blood, sera or tissue samples for the entire life of the animal. Nevertheless, the detection of antibodies in a field sample that resulted PCR weak or negative, is not only an indicator of the presence of survivors from acute or subacute infections. Animals infected with non-HAD and low virulence strains seroconvert after the first week of the infection even in absence of clinical signs or viremia. The antibodies are easily detected by IPT and ELISA, reaching antibody levels >1:160,000 after one month and are maintained over the time (Gallardo et al., 2015c, 2018, 2019a; Leitao et al., 2001; Sánchez-Cordon et al., 2017; Sánchez-Vizcaíno et al., 2015). The relative low percentage of non-HAD viruses isolated within the EU could come to the fact that these non-HAD viruses are more difficult to isolate than HAD viruses since the viraemia they cause is sporadic and virus is isolated in small amounts from the organs. Infection of wild boar with these attenuated non-HAD isolates may account for the seropositive animals detected in thefield in absence of clinical signs and viremia. These data emphasize the fact that early detection based only on clinical signs and ASFV genome detection is not an efficient approach for the control of ASF in the current epidemiological situation in Europe. It is likely that the European wild boar is being endemically infected in certain regions becoming a recurrent source of infection to other wild boar, but also to domestic pigs. Since each animal could be at a different stage of the infection, sub-clinically ASFV infected wild boar or domestic pigs can be detectable if both virus and antibody detection tests are used, for examining transient viremia and the presence of anti-ASFV-specific antibodies. A positive test for the presence of the virus indicates that the tested animal was undergoing infection at the time of sampling. On the other hand, a positive ASFV antibody test indicates an ongoing or past infection, where the animals have recovered (and may remain seropositive for life; tab. 3).

Table 3. Interpretation of the ASF diagnostic results.

Assay	Result	Probable scenarios
PCR Ab-ELISA IPT	WEAK $_{(Ct>35)}$ NEGATIVE NEGATIVE	Animal has been recently infected and it has not yet seroconverted (< 7 days). Clinical signs cannot be evident.
PCR Ab-ELISA IPT	POSITIVE NEGATIVE POSITIVE	Animal has been recently infected, develop clinical signs and is initiating the seroconversion (7-10 days)
PCR Ab-ELISA IPT	POSITIVE POSITIVE POSITIVE	Infection in course. Animal is still viremic with clinical signs and has already seroconverted (>10 days). Reinfection of an animal with preformed antibodies from a previous infection (survivor)
PCR Ab-ELISA IPT	WEAK $_{(Ct>35)}$ or NEGATIVE POSITIVE POSITIVE	Past infection. Animal has recovered from acute or subacute infection and did not present clinical signs. Animal infected with attenuated strain (with or w/o clinical signs). Reinfection of an animal with preformed antibodies from a previous infection (survivor).

Final remarks

In conclusion, control-eradication programs in areas with a clear endemic tendency should be reviewed and updated and should include parallel routine laboratory monitoring together with the regular clinical inspection. The use of the most fitting diagnostic tools combining both ASF virus and antibody detection will improve the efficacy of disease control measures, regardless of the nature of the circulating ASFV strains (Arias and Sánchez-Vizcaíno 2002, 2012; Gallardo et al., 2015a, 2019b). An accurate evaluation of the results of the serological and virological tests must be carried out, considering all the clinical and epidemiological findings, in the framework of the enquiry to be carried out in case of suspicion or confirmation of ASF.

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African swine fever and experiences from affected countries

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Introduction

Although only two years have passed since the last IPVS congress in Chongqing (China), the global swine industry has been completely transformed by the emerged threat of African swine fever (ASF). The introduction of ASF to South East Asia, especially to China, has altered perceptions of ASF. On one hand, the massive scale of the outbreak in the People Republic of China (PRC) has given the perception that the situation in many countries is not so severe. On the other, China's huge effort in ASF outbreak mitigation has dramatically increased attention to the ASF problem and the number of research projects in the country: in 2019 alone, more ASF diagnostic kits have been developed and applied to the field conditions than in previous over 100 years. These new approaches to ASF diagnostics and surveillance need to be assessed.

Several recent publications suggest that commercial ASF vaccines will be soon available. Now is the time to start discussions with the swine industry on possible strategies for ASF vaccination and the future role of ASF vaccines as a measure for ASF.

2018-2019 brought a little new to ASF epidemiology. The experience of affected countries confirms again and again that low biosecurity level of pig farms continues to play the most crucial role in introducing and spreading of ASF to new areas. In China, the increasing risk of ASF introduction and the tremendous consequences were evident for many years prior to the outbreak.

The last two years did indicate a paradox of biosecurity issues. All agree that to reduce ASF risks, biosecurity across the industry needs to be improved, including a reduction in the number of backyard farms. However, we saw very few examples of countries sufficiently investing in pig production systems prior to ASF introduction. Still, adequate changes in the pig industry began only after ASF spread within the country. Such reactive policies only multiply the costs of upgrading the biosecurity infrastructure of the industry, including losses from ASF itself. From the epidemiological point of view, ASF is much easier to control and eradicate, especially in the domestic pig population, than are other significant swine diseases - PPRS for example. But the number of effort and efficacy to control ASF depends mostly on economic drivers in the country and/or region.

Finally, ASF reporting needs to consider the potential for transboundary spread of ASF through and in wild boar. Recent situations in Indonesia and Denmark, where pig carcasses floating on the sea reached the coasts of ASF-free countries, did not affect the ASF free status of those countries. This kind of cases needs to be foreseen by legislations. Given the situation, it might be effective to provide an incentive for countries to stimulate them to be transparent when reporting potential cases of ASF.

Overview of Epidemiological situation on ASF in the Russian Federation

African swine fever outbreaks have been reported in Russia since 2007. Despite all measures taken, 1527 outbreaks have been detected from the initial outbreaks in Russia to the end of 2019 - including 912 in domestic pig populations and 615 in wild boars. Recently, some measures have been taken in the Russian Federation to reduce the morbidity from ASF. A decree by the Government of the Russian Federation has been issued to enact a plan for ASF control. The veterinary rules and regulations have been updated by the order of the Ministry of Agriculture of the Russian Federation. These rules include requirements for keeping pigs, diagnostics, preventive and control measures, and other restrictions aimed at preventing the spread and elimination of ASF outbreak. The main requirements presented in published documents include the organization of full cycle of pig production, ensuring a high level of biosecurity at all enterprises on breeding and slaughtering of pigs and processing of pig production, traceability of animals and products, reduction the density of wild boar population in the threatened zones, and mandatory reporting to the state veterinary service about all cases of suspicion of ASF. The electronic traceability system “Mercury” of livestock products has been launched. Despite these recent mitigation efforts in Russia, the foci of ASF have continued to expand.

In 2016, in the territory of the Russian Federation, ASF was detected in domestic pigs in 215 localities (239 foci) in 26 of 85 regions of Russia, and there were 527 cases of ASF in wild boar (65 locations, infected objects) in 17 regions. According to the Federal Service for Veterinary and Phytosanitary Surveillance, the economic losses were estimated at 24.8 million Euros. In 2017, 150 affected locations (165 foci) were notified in domestic pigs in 23 regions of Russia, and there were 330 cases of ASF in wild boar (102 affected locations and 11 foci).

In 2018, the number of outbreaks in domestic pigs reduced: 56 affected locations and 21 infected objects in 18 regions of Russia and 143 cases in wild boar. However, in 2019 the situation in domestic pigs got worse (79

notifications). It was caused mainly by ASF outbreaks emerged in the Far East, in the area bordering with China. Primary outbreaks were registered mostly in the backyards of the Amur region, and later in Primorsk Territory, Khabarovsk region, and the Jewish Autonomous Region. ASF has been detected both in domestic and wild bigs populations. According to the regional veterinary services, the primary causes of rapid ASF spread in Russian territories bordering with China include the low level of biosecurity of pig farms, the high number of completely unprotected backyards and high density of wild boar.

At the Federal Research Centre for Virology and Microbiology, we sequenced the genomes of ASFV isolates from this area. Sequenced genome regions of the isolates were identical to those from ASFV isolates from Belgium, China, and Georgia available in Genbank.

It is also worth mentioning that in some regions in wild boar of the same area the period between ASF notifications is more than 6 months, and this phenomenon cannot be explained by the reintroduction of the virus.

Since the beginning of the epidemic, another serious problem related to ASF spread is the uncontrolled movement of pork products contaminated with the virus (or its genome) from affected (which are not always declared by the owner to the veterinary service timely) pig farms to long-distant areas. The presence of ASFV DNA can be considered as an indicator of the efficiency of the ASF control system in a country. In 2019 in the Russian Federation, there were 2 cases when ASFV genome DNA was spread widely by pork products. In the first case from (October 2019), the batches of pork products produced from infected pigs were shipped from Kaluga region to 39 regions of Russia. In another case, bathes of pork products delivered from infected pigs and containing ASFV DNA were shipped from the Stavropol region to other 18 regions of Russia.

Conclusions

After more than ten years of African swine fever virus circulation in Russia, the national pork industry is in very good shape. The latest USDA forecasts suggest that Russia’s domestic pork production is set to increase by 2.7% year-on-year in 2019, to finish at 3.24 million tonnes (cwe). Consumption is predicted to be slightly above this during 2019 at 3.31m tonnes (cwe).

In 2020, the USDA anticipates that Russia’s domestic production will grow to equal its domestic consumption levels. Both are forecast to reach 3.33m tonnes in 2020. The domestic pig herd in Russia is forecast to reach 23.6 million head during 2019, making it one of the largest pig herds globally.

Past years confirm that ASF is a "trade" disease, and for many countries without export ambitions, the role of ASF is overestimated.

Based on the information from the OIE reporting system, there have been no recent cases of ASF reported in Transcaucasian Countries and Belarus. Recently, the Czech Republic and Estonia declared their freedom from the disease in wild boar and domestic pig populations. Based on that, we could conclude that the eradication ASF can be achieved even without a vaccine.

Recent studies have shown that all the field strains isolated in the Russian Federation in 2019 induce in bioassays the same high level of pathogenesis and acute (up to 21 days, including incubation period) course of the disease in domestic wild pigs as Georgia 2007 reference strain does. Given such short length of the course of the disease, two possible explanations for the phenomenon of 6-months interval between detections in the same wild boar subpopulation can be proposed: 1) unknown reservoirs of the virus in the environment or 2) the weakness of the surveillance system and hidden circulation of the virus in this area.

As some recent surveys have shown, there are still many knowledge gaps about ASF. Many scientific projects and scientists all around the world are working actively to fill in these gaps. To consolidate these research efforts the **Global African Swine Fever Research Alliance** (GARA) was established in 2014. GARA has a mission of establishing and sustaining global research partnerships that will generate scientific knowledge and tools to contribute to the successful prevention, control and where feasible eradication of African Swine Fever (ASF). GARA aims to expand ASF research collaborations worldwide and maximize the use of resources and expertise to achieve its five strategic goals:

1. To facilitate research collaborations and serve as a communication gateway for the global ASF research community.
2. To conduct strategic research to increase our understanding of ASF.
3. To develop the next generation of control measures and strategies for their application.
4. To determine the social and economic impacts of the new generation of improved ASF control
5. To provide evidence to inform the development of policies for the safe trade of animals and animal products in ASF-endemic areas.

Additional information on the GARA Project and the work of the Alliance can be found on the website: http://www.ars.usda.gov/GARA.

ASF, the main threat for the global swine industry

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The disease

African swine fever (ASF) is one of the most important infectious disease of swine. This disease causes greater sanitary, social and economic impacts due to its high mortality rate in domestic pigs and restrictions on the pig and pork trade. It is not a zoonotic disease, which limits its impact on public health. ASF must be notified to the World Organization of Animal Health (OIE). There is not a commercial vaccine or treatment against ASF at present, because of that, its control is based on early detection and the application of strict sanitary measures and of biosecurity.

The African swine fever virus (ASFV) is a complex, large, enveloped DNA virus with icosahedral morphology. It is classified as the only member of the Asfarviridae family, genus Asfivirus. It presents a huge genetic and antigenic variability existing 24 genotypes based on differences observed in the C-terminal region of the gene vp72. Genotype II is current circulating in Eastern Europe since 2007, in the European Union (EU) since 2014 and in Asia since 2018

ASFV can infect all members of the suidae family. Domestic pig (*Sus scrofa domestica*) and wild boar (*Sus scrofa*) show a variety of clinical presentations: peracute, acute, subacute, chronic or subclinical (Sánchez-Vizcaíno & Arias, 2012). In Africa, wild suid species including warthogs (Phacochoerus spp.), bushpigs (Potamochoerus spp.) and the giant forest hog (Hylochoerus meinertzhageni), develop asymptomatic infections and act as ASFV reservoirs. ASFV also replicates in soft ticks of the *Ornithodoros genus* (*Ornithodoros moubata* in Africa and *O. erraticus* in the Iberian Peninsula), which act as biological vectors and reservoirs (Jori et al., 2013)

The transmission of the disease can occur through direct contact with secretions or excretions of infected animals, such as blood or feces. There may also be an indirect transmission through ingestion of meat or meat products from infected animals or through contaminated feed or fomites (e.g., swill feeding, vehicles, clothing, boots) (Sánchez-Vizcaíno et al., 2015). Finally, its transmission through the biological vectors (soft ticks of the genus *Ornithodoros*) or mechanical vectors (flies of the species *Stomoxys calcitrans*). (Mur et al., 2017)

The clinical signs vary depending on the species affected, the virulence of the virus, the infective dose and the route of infection among other factors. The genotype II, athat currently circulates in Europe and Asia, is highly virulent and induces an acute form of the disease in domestic pig (Gabriel et al., 2011)

The acute form is characterized by the appearance of signs such as high fever, anorexia, erythema, hemorrhages in skin and internal organs, weakness, abortions, vomiting or diarrhea In these cases the mortality is close to 100% and usually occurs between 4 and 9 days after infection (Sánchez-Vizcaíno et al., 2015). However, the existence of wild boar survivors of genotype II infection, has play a leading role in the epidemiology of ASF in Europe, being involved in the maintenance of the disease in endemic areas (Armenia, Azerbaijan, Georgia, Russian Federation, Belarus and Ukraine) and in its introduction and dissemination within the countries of the European Union. Other genotypes of a lower virulence may give subacute, chronic or inaparent clinical forms, in which mortality rates are lower and recovered animals can act as potential carriers of the virus.

The diagnosis must always be confirmed with laboratory tests in order to perform a differential diagnosis with bacterial sepsis or other hemorrhagic diseases such as classical swine fever (CSF) that have similar clinical conditions. All techniques currently used to diagnose ASF have been fully validated, showing high sensitivity and specificity to detect antigens and antibodies against known genotypes, allowing the correct diagnosis of ASF in all possible epidemiological scenarios (Sánchez-Vizcaíno & Mur, 2013)

There is currently no commercialized vaccine or treatment for ASF. The measurements of prevention and control are based on early identification and diagnosis of the disease followed by the establishment of strict sanitary measures, so the development of a effective and safe vaccine is one of the most promising bets for control and PPA eradication (Arias et al., 2018)

Brief history of the disease

The ASF was discovered by Montgomery in Kenya in 1921, as an acute hemorrhagic fever what caused high mortality in imported European pigs. Since then, the disease has been reported in most sub-Saharan African Countries. The disease was entirely restricted to the African continent until 1957, when there was a first transcontinental spread to Portugal, near Lisbon, with an outbreak with mortalities close to 100%. The disease was rapidly controlled, however,

after an epidemiological silence of three years, in 1960 the disease was notified again in Portugal, spreading in the following years throughout the Iberian Peninsula, several European countries (France, Italy, Malta, Belgium and Holland) and Latin American countries (Cuba, Brazil, Dominican Republic and Haiti). However, all these foci were eradicated except for the island of Sardinia where the disease remained endemic until 2019.

The years following its eradication were of apparent calm for Europe while the disease continued to spread and becoming increasingly important in Africa, affecting many countries previously free of the disease. This expansion facilitated a new spread to the European continent, in 2007, specifically in the Georgian port of Poti, through contaminated meat residues transported in ships from Africa and used to pig feed. All the control measures implemented below were unable to stop its spread and the disease began its unstoppable expansion across the European continent with a slow but steady geographical advance. The disease already affects 18 countries of the European continent (Georgia, Armenia, Russia, Azerbaijan, Ukraine, Belarus, Lithuania, Latvia, Estonia, Poland, Moldova, Czech Republic, Belgium, Bulgaria, Hungary, Romania, Slovakia and Serbia), 11 of them belonging to the European Union. In 2018, the virus reached the Asian continent for the first time in history, where it has spread rapidly and unstoppably, currently affecting 12 countries (China, Mongolia, Vietnam, Cambodia, North Korea, Laos, Slovakia, Myanmar, Philippines, South Korea, East Timor, Indonesia), (OIE, 2020).

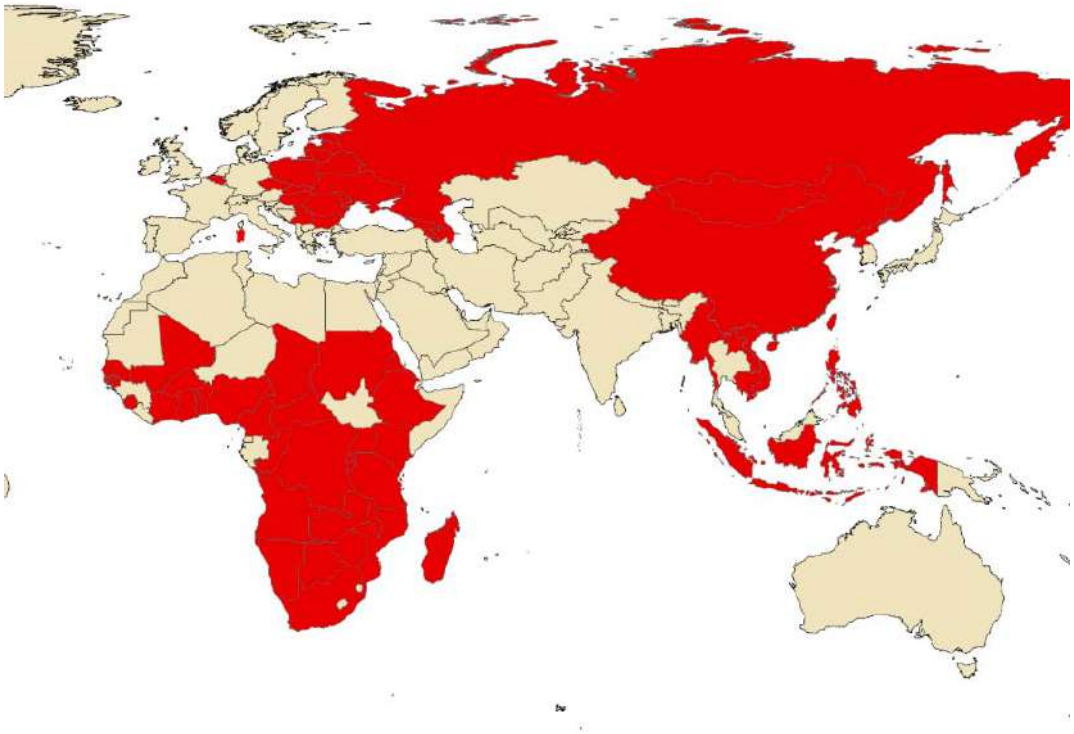

Figure 1. Countries affected by African Swine Fever (OIE, 2020). Update in January 2020.

Current epidemiological scenarios

Africa. The African continent gives its name to the disease since it was where it was first described in 1921 by Montgomery and since then, the disease has been reported and remained endemic in many countries. In Africa, two different epidemiological scenarios are described.

The first one occurs in southeast Africa, where the disease is endemic. There is a very complex epidemiological cycle characterized by coexistence of wild suid species such as warthogs (*Phacochoerus* spp.), bush pigs (*Potamochoerus* spp.) and giant forest hogs (*Hylochoerus meinertzhageni*), *Ornithodoros moubata* soft ticks and domestic pigs. The wild suidae are distributed in abundant way in Southeast Africa and have the peculiarity of being tolerant to the ASF virus, since they are able to maintain very low blood virus levels that are not enough for the development of the disease, in such a way that they act as asymptomatic carriers of ASF and reservoir of the virus. Also the virus infects and replicates in soft ticks of the genus Ornithorodoros Moubata which are the only known natural arthropod hosts of the virus and act as reservoirs and biological vectors in the transmission between wild suidae and domestic pigs. In this way, the three cycles of transmission have been confirmed in this region: two cycles that involve soft ticks (wild suid-tick and domestic pig-tick) and a cycle involving circulation among domestic pigs. In this area the

24 genotypes described to date have been identified.

The second scenario is less complex and one occurs in countries in west and central Africa. Although many countries in this area are at present endemically infected, the history of ASF in that region is more recent and likely due to introductions that occurred mainly in the late 1950s and again in the 1990s. To date only genotype I has been recovered in this area. In this epidemiological cycle, only domestic pigs participate and the transmission is due to contact between infected and susceptible animals, as well as the consumption of contaminated pig products (swill feeding). In the center of the African continent, specifically in Malawi, the transmission of the virus in domestic pigs through *Ornithodoros porcinus* has also been described.

Europe. In Europe, the disease affects both domestic pig and wild boar. However, the high density of wild boar, which play a fundamental role in the spread and maintenance of the virus acting as reservoirs and as a source of introduction of the disease through connected natural landscapes and spreading the disease by means of contact with domestic pigs.

As we have explained above, it is not the first time that the disease affects the European continent. When the disease affected Portugal and Spain, an epidemiological cycle was observed with soft ticks of the genus *Ornithodoros* of the species O. *erraticus*. However, the presence of these ticks has not been demonstrated in the countries currently affected and therefore their involvement in the current epidemiology could be nil.

At present we can distinguish three epidemiological scenarios in Europe in function of the host affected predominantly. The first one occurs in the countries belonging to the European Union affected (except Romania, Bulgaria and Slovakia), where more than 90% of ASF cases in Europe are attributed to wild boar with sporadic outbreaks in domestic pig farms. In these countries, control strategies should be aimed at controlling wild boar populations and increasing biosecurity in domestic pig farms to avoid contact with wild boars.

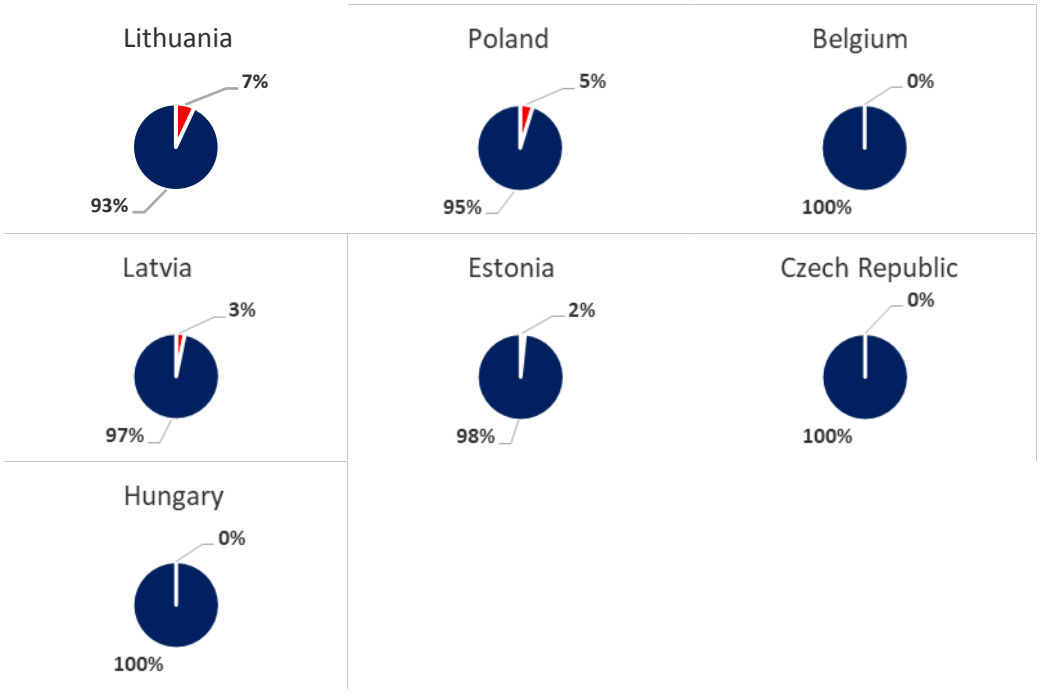

Figure 2. Percentage of host affected by country, differentiating between domestic pig (red) and wild boar (blue), (OIE, 2020). Update in January 2020.

The second epidemiological scenario occurs in the countries of Eastern Europe (Armenia, Azerbaijan, Belarus, Georgia, Moldova, Serbia, Russia and Ukraine), Bulgaria, Romania and Slovakia. 40% of the reported cases are in wild boar and 60% in domestic pig. One of the main differences in this epidemiological scenario is the existence of a large number of family or backyard farms with poor levels of biosecurity, which are usually located in areas with wild boar, increasing the risk of virus transmission. In addition, in many of them, the animals are fed with contaminated food scraps. This practice is known as "swill feeding" and is currently prohibited in the European Union since it is an important route of disease transmission.

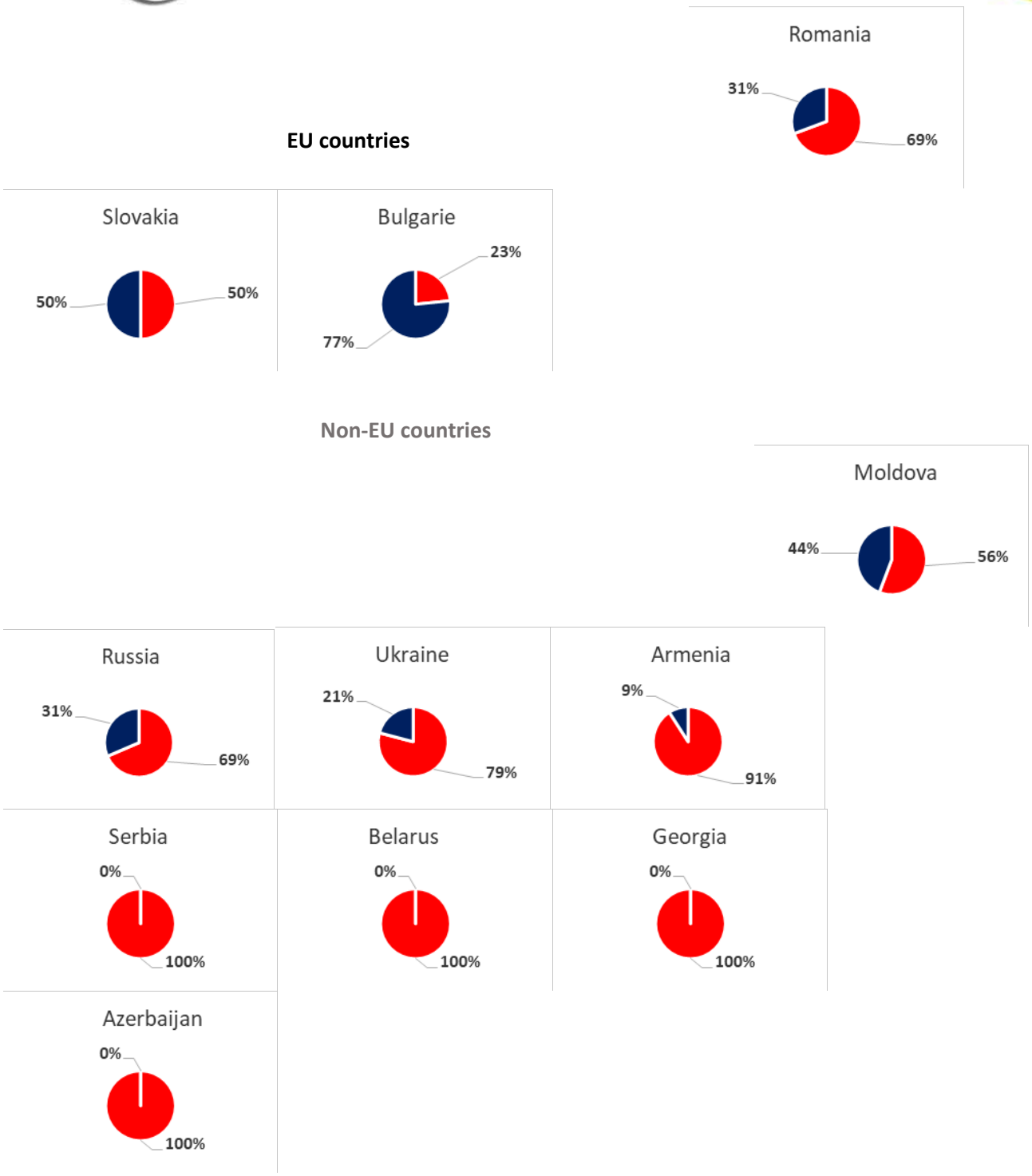

Figure 3. Percentage of host affected by country, differentiating between domestic pig (red) and wild boar (blue), (OIE, 2020). Update in January 2020.

The last epidemiological scenario in Europe is located in the Italian island of Sardinia, where the disease has been endemic since 1978. The endemic factors appear to be poor biosecurity conditions in farms, high densities of wild boar and local and, specially, traditional farming practices such as raising non-registered domestic pigs (free-ranging pigs). This free-ranging pigs, known as “brado”, are a virus reservoir and provide a route of transmission between domestic pigs kept in backyards and wild boar populations. (Jurado et al., 2018). Several control and eradicaton programmes, which included the prohibition of brado in 2012, have achieved to reduce the outbreaks and that there is no cases of ASF on the island during the year 2019.

Figure 4. Example of a camera trapping image showing direct interaction between a free-ranging pig and wild boar. (Cadenas-Fernández et al., 2019)

Asia. The Chinese authorities reported the first outbreak of ASF in Asia in August 2018, at a domestic pig farm located in Shenyang city. This was a spectacular breakthrough for the disease since China is the world's leading producer of pork, with about half of the pig population worldwide. Epidemiological studies indicated that the outbreak could have been due to the importation of infected piglets or “swill feeding”, common in Asian backyard farms. In 2019, the disease has spread considerably faster than in other epidemiological scenarios and 11 countries of the Asian continent are currently affected (China, Mongolia, Vietnam, Cambodia, North Korea, Laos, Myanmar, Philippines, South Korea, Timor-Leste and Indonesia). In these countries, 100% reported cases are in domestic pigs and the main causes are poor biosecurity measures applied on farms and "swill feeding". In addition, the notification of outbreaks in very separate places suggests the transport of infected animals or pork is facilitating this rapid spread. Therefore, control strategies in this continent should be focus on improving biosecurity and on the prohibition of "swill feeding”. The lack of compensation to the affected farmers is another important risk factor for the control of the disease as well as the partial depopulation carrier out in some farms.

Another notable aspect to consider in the Asian continent is the estimated high density of wild boars in some areas, even higher than in the European continent. This aspect is of great interest, since as in the European scenario, the wild boar could play a very important role in the endemism and maintenance of the ASF in Asia. So far, outbreaks reported in wild boars are very low compared to domestic pig, with the majority being concentrated in South Korea, affected since September 2019.

Current situation

The current situation of African swine fever has acquired a pandemic dimension and suggests an imminent risk for world swine production. The disease has spread to 47 countries on three continents. Currently the 77% of total swine population is already living in an infected area. China, the main pig producer, with about half the head of pigs from around the world, has lost more than 37% of its porcine population. Globalization has increased the risk of ASF being introduced into free areas. The main risks of ASF spread are the continuous movement of infected wild boar populations, the poor levels of biosecurity in farms and the illegal movement of live pigs and infected pig meat and products coming from infected areas.

A point to highlight in the control is the human factor, main responsible for the spread of the virus to new regions, with practices such as feeding of pigs with contaminated products (“swill feeding”), movement of infected pigs

or products and in general, the lack of biosecurity measures. Moreover, we must pay close attention to wild boars. The density of this animal in Europe and Asia is very high and this host is very susceptible to virus infection. In this way, the wild boar is playing a very important role in the maintenance and endemism of the disease, as observed in Europe.

The control of this disease is very complex and requires a great effort at all levels (better communication about the disease, field-administration-research). One of the great challenges is the lack of treatment or commercial vaccine. At this point, vaccination is considered to be the most efficient strategy and solution for emerging infectious diseases. Regrettably, attempts over many years to develop a vaccine have failed. Last year new several vaccines prototypes for domestic pig and wild boar have been described with very promising results (Barasona et al., 2019).

In this critical situation, A EU project, "VACDIVA", has been financed with 10 million euros by the European Union with the objective of develop an effective, safe and DIVA vaccine against ASF over the next four years (VACDIVA H2020 Grant ID: 862874). The research works involve two international ASF reference laboratories, both of them Spanish: the Centre for Veterinary Health Surveillance (VISAVET) of the University Complutense and the Animal Health Research Centre (CISA-INIA) of the National Institute for Agricultural and Food Research and Technology. Additionally, six national UE laboratories (from the 10 countries currently affected by ASF), four prestigious ASF research centres and two leader companies in vaccine´s production and ASF diagnostic kits. It also has the collaboration of Chinese, Russian and African laboratories, which will provide very useful support for the project. Finally, the active participation of pig producers, agricultural associations, hunting associations and international agencies, will expand the impact of the activities of communication, dissemination and training of the project.

The project, which will last the following four years, have three objectives:

- To provide an effective, safe and DIVA vaccine(s) for wild boar and domestic pigs already for registration.
- To develop DIVA test to allow an accurate monitoring of the effectiveness of the vaccine.
- To design ASF control an eradication strategies in different epidemiological scenarios worldwide and test the pilot

vaccine in real environments (including buspigs and warthogs).

Finally, the situation described is affecting very much the global swine industry, meat prices are increasing around the world and the pig production is trying to address these changes. A personal view of these future changes will be also discussed in this presentation.

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Recognizing African swine fever disease and evolution

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Introduction

African Swine Fever (ASF) is a devastating highly contagious disease affecting suids. It is caused by a big complex virus, the African swine fever virus (ASFV), that can spread very rapidly within the pig populations by direct or indirect contact. This virus can also become endemic in feral or wild suids, and the transmission cycles existing between these animals and the *Ornithodorus spp.* ticks, the biological vector, complicate control and eradication programmes.

The domestic pigs (DP) and Eurasian wild boars (WB, *Sus scrofa scrofa*) are susceptible to the virus infection showing a variety of clinical symptoms. In contrast, a tolerance to virus infection is observed in wild suids in eastern and south sub-Saharan Africa, as while infected they do not show clinical signs or lesions. Acute forms of ASF in susceptible DP and WB are commonly observed at the first stages of the virus introduction into free areas. These animals usually show extensive congestive-haemorrhagic pattern and functional disorders in the digestive and respiratory system, with high mortality rates that may reach up to 95-100%. Other clinical forms of the disease can be also observed after a time of the virus presence in a region, including asymptomatic courses (Arias &Sánchez-Vizcaíno, 2002).

ASF is considered a global animal health priority. Currently it is present in more than 26 countries in Sub-Saharan Africa, in 18 countries in Europe (importantly in eastern European countries) and 11 countries in Asia. This also includes China with at least 32 provinces / autonomous Regions / municipalities / special administrative regions affected (FAO, February 2020), and Vietnam, with all provinces and municipalities struck by the disease. Its rapid dissemination in Asia since 2018 has resulted in major problems for the swine industry with huge socio-economic consequences. The disease presence in affected countries provokes significant problems at local, regional and global level, and great economic losses by livestock with closure of export markets.

ASF was maintained confined in Africa since the first description in 1921, jumping out the continent three times towards Europe, particularly Portugal twice (1957, 1960) and forty-seven years later Georgia in 2007. When ASF arrived originally to Europe it was spread to Spain (1960-1995) affecting later on to several European countries (in the period 1964-1993), as well as four countries in the Americas (period 1971-1983). Since 1999, the disease was restrained in sub-Saharan Africa and in the island of Sardinia (Italy) until 2007. During this period, several groups of scientists mainly those in Europe, as well as those belonging to the European Union Reference Laboratory (Madrid, Spain) and to the OIE Reference laboratories, in Madrid (Spain), Pirbright (UK) and Onderstepoort (South Africa), kept on working in a wide spectrum of ASF gaps related to disease maintenance, virus transmission, immune mechanisms involved in protection, vaccine development, diagnosis, virus-host interactions, reservoirs, carrier state, and many other epidemiology and control issues. Despite this, more resources in research and education of vets. farmers and producers were needed. However, ASF was not considered important enough, and in spite of the fact of the past cases and its serious consequences, the potential for an ASF re-emerging was underestimating. Therefore, at the time the disease appeared in Georgia, there was a significant unresolved number of gaps, a lack of knowledge about the disease, and a wrong perception about the scale of the problem that ASF and its causative clever virus could cause. What is more, faced with the immediate devastation of peracute and acute ASF, it was not given much thought to the potential dangers of the subacute and chronic infections that were soon to arise. Even this, and because the high virulent virus moving on causing mortality rates near to 100%, at the beginning it was initially consider by some as quite similar to the acute disease caused by other important haemorrhagic disease: Classical swine fever (CSF), which exhibits similar clinical symptoms than ASF. However, nothing could be further from the truth. ASF and CSF are both quite different diseases. ASF is much more complex to combat because of the complex epidemiology, the biological reservoirs, and the lack of neutralizing antibodies and despite the attempts, the lack of a commercial vaccine available. CSF and ASF viruses are completely different: CSFV is an RNA virus of 12,3 kbp. producing around 12 mature proteins, two of them involved in the induction of neutralizing antibodies, which facilitate the availability of a safe effective vaccines (Zhang et al., 2011). By contrast, ASFV is a large complex DNA virus of 170-193 kbp, and , more than 100 polypeptides are induced in the infected cells, with capacity for up 150 (Arias et al., 2017) and despite a high amount of antibodies the virus produces in the infected animals, these are unable to neutralize completely the virus, and this is one of the reason why in spite of the efforts, there is not a vaccine to date.

This review aims to give an updated information about the disease knowledge and recognition, emanating from both the past and the current epidemics, in the different scenarios, pointing out some important issues that matter to veterinarians, producers, hunters and veterinary services. To get success in control and eradication programs is necessary to avoid the solely idea that ASF must give rise to severe lesions and have a mortality rate closely

approaching 100%. Based on scientific studies performed in the past epizooties in Europe, and in the Americas, and more recently in the current epidemic in Europe, we give a detailed description of a variety of forms of reduced mortality that can be also present in variable percentage, and therefore arising survivors, recovered and immune animals that play a role in virus transmission, spreading and disease maintenance.

ASF virus (ASFV): a rather unique pathogen

ASFV naturally infects and replicates in macrophages, key actor cell in arising an effective immune response against foreign invaders such as ASFV (Arias et al., 2017). The major components of the viral capsid have been also identified as the most antigenic proteins which are responsible for the production of specific antibodies in the infected animal after a natural infection. However, regardless of the usefulness of these proteins as sero-diagnostic targets, they are not sufficient to develop a protective response based on specific neutralizing antibodies nor developing antibody-cell mediated protection against virus infection. Nevertheless, a great amount of specific antibodies against ASFV can be detected from the second week of infection by laboratory diagnostic techniques (Gallardo et al., 2019a). This feature makes ASF specific antibodies good markers to point out the disease presence. ASF specific antibodies are very useful for the detection of survivors and recovered animals, since stay detectable for months and up to years. The specific antibodies are partly responsible for delaying the appearance of clinical signs and reducing viremia levels. However they are not good markers for the detection of acute infection, which is major in European and Asian affected countries, since a large percentage of infected animals usually died during the first 6-11 days post infection, before ASFV specific antibody appearance.

Due to the fact that not fully neutralizing antibodies are induced during the virus infection, it has not been possible to perform a universal classification of ASF virus isolates based on serotypes. Instead, since the beginning of this century it has been internationally accepted to classify the ASF viruses following genotyping. This is based on sequence analysis of a specific fragment of the gene encoding the major viral protein p-72. Up to 24 genotypes have been described so far, all of them coexisting in Africa (Achenbach et al, 2017; Quembo et al. 2017). Two genotypes (I and II) went outside Africa so far: the genotype I emerged in Portugal in 1957 and 1960, further spreading to Europe and the Americas. Besides the genotype II reached Eastern Europe (Georgia) in 2007, being the causative virus involved in the current epidemic wave by spreading towards west and east territories, getting Asia in 2018. Although belonging to the same genotype II, the molecular analyses of specific variable genome regions of the European and Asian isolates have identified a range of genetic variants as a result of the natural evolution of the virus. However, at the level of the whole genome, the sequence homology among isolates from different regions is greater than 99.9%, which is in the range of the typical genome stability of DNA viruses (Magolokin et al, 2012; Gallardo et al., 2014; Ge et al., 2018; Bao et al., 2019).

It is important to point out that the term "*genotype*" is a molecular concept, used to establish relationship between viruses and therefore to trace the origin of outbreaks. However, the molecular characterization by genotyping doesn´t correlate or identify with virulence or any specific clinical form of the disease. Instead, the ASF disease presentation is produced by multiple complex factors, including i) the specific virus strain affecting, characteristics, dose and route of infection; ii) the presence of the hosts and transmission cycles (domestic pigs, wild suids and *Ornithodoros spp*.), the immunological and genetic characteristics of the host, the virus-host interaction, and the role that each actor plays in a specific scenario, in a certain period of time. In addition iii) the environment and human factors, which also play a role in the disease expression, since both elements could influence virus dissemination and maintenance of certain virus strains.

ASFV is highly resistant in the environment and can persist in blood and tissues after death of the infected animal. We should be in mind, the cannibalism of carcasses by WB has been demonstrated and it can contribute to virus transmission in wild suids in the forest. In addition, massive environmental contamination may result by the secretion and excretions of these infected animals, including bloody diarrhea, or if blood is shed during necropsies. These factors, among others make more difficult the control of ASF in wild suids. Importantly, the virus can be maintained alive for long periods of time in frozen and chilled blood or organic infected material. The virus persists as infectious in sera or blood stored at room temperature for 18 months and for up to 15 weeks in putrefied blood (EFSA 2010). It has been also established that virus can remain infectious in contaminated pens for several days and up to several weeks in pig faeces, urine or slurry (EFSA 2010).

In meat products, the virus may persist for several weeks or months in frozen or uncooked meat. Nevertheless, it has been scientifically demonstrated that Spanish cured pig-meat products, such as Serrano hams and Iberian hams and shoulders were free of viable ASFV by day 140 and Iberian loins by day 112 – before the cured period process-(Mebus et al. 1997). In cooked or canned hams, no infectious ASFV has been found when these products were heated to 70°C. Infectivity of ASFV is lost by 110 days in chilled deboned meat, bone-in meat, or ground pork and after 30 days in smoked deboned meat (EFSA, 2010).

On the contrary ASFV is very sensitive and inactivated by heat treatment at 60ºC for 20 minutes or 1h a 56ºC, and by many lipid solvents and commercial disinfectants (EFSA, 2010).

The Host

The epidemiology of ASF is very complex and varies greatly. Domestic pigs and Eurasian wild suids are susceptible to ASFV infection. In contrast African wild suids (warthogs, bush pigs and Giant Forest hogs) develop asymptomatic infections. The molecular factors involved in this tolerance have not been yet determined. The fact of this asymptomatic wild suids presence in eastern regions of Africa makes eradication very difficult in the region. African wild suids established an ancient virus transmission cycle involving soft ticks. Both act as natural reservoirs in Africa (Detray, 1957; Penrith & Vosloo, 2009). The infection of African wild suids usually results in low virus titers in tissues and low or undetectable viremia. These levels of virus are sufficient for transmission to domestic pigs through tick vectors, but usually not by direct contact between animals. The soft ticks *Ornithodorus spp* are ASFV biological reservoirs, and transmission vectors. They are present in eastern and southern parts of sub-Saharan Africa, particularly *O. moubata*, the African tampan. In a similar way, *O. erraticus* exists in the western regions of Iberian Peninsula and they played a role in the epizooty occurred in 1960-1995 (Arias and Sánchez-Vizcaíno, 2002; Sánchez-Vizcaíno and Arias, 2019). However it has not been established a potential role of soft ticks in the current ASF epizooty in Europe.

Several *Ornithodorus* species experimentally tested seem able to transmit ASFV, including, *O. porcinus, O. coriaceus. O. turicata and O. savignyi* (EFSA, 2010; Groocock, et al.,1980; Hess et al., 1987; Jori et al., 2013; Mellor & Wilkinson, 1985). Other species of soft ticks have been identified in regions of North and South America and should be considered to potentially harbor and transmit ASFV (EFSA 2010; Donaldson et al., 2016). In the absence of viraemic hosts, *O.* ticks can allow ASFV infection to persist for more than 5 years in a region (Boinas et al., 2011). The geographical distribution of *Ornithodorus.* spp in many regions of the world has not been yet well elucidated. It should be taken in mind the presence of *Ornithodorus spp* in the Americas could potentially have a role in the continent, in case of an ASF incursion.

Several studies in Eastern part of Africa have revealed a complex epidemiological situation in which local breeds of domestic pig seem to show greater tolerance to ASFV that can favours endemicity and spread of the disease (Atuhaire et al., 2013; Uttenthal et al., 2013). These “Tolerant" indigenous pigs in Mozambique and in Kenya, and the presence of ASF sero-positive animals in regions of Malawi might reside in the immunogenetics and genetic characteristics of the indigenous pig populations but can also be related to population immunity (Haresnape et al. 1985, 1987; Gallardo, et al., 2012; Okoth et al, 2013). Besides this, virus evolution towards moderate virulent forms in an area could be also contributing to the presence of asymptomatic pigs.

Considering all the three actors, domestic pigs, wild suids and the *Ornithodorus spp*., as well as the different susceptibilities that domestic and wild suids may show, together with their coexistence in the scenarios and, taking into account their interactions and virus transmission cycles, that will be dependent of many factors (such as the environment -agricultural areas, forest, vast monoculture plantations- as well as the human factors), all these items reflect the great complexity that ASF represents and the difficulties to prevent its spreading.

The Disease

ASF is not associated with pathognomonic lesions, and clinical signs observed in its acute or chronic forms may be similar to other haemorrhagic diseases such as classical swine fever, salmonellosis or erysipelas. The virus is commonly transmitted when unexposed pig populations (domestic or wild) have direct or indirect contact with blood, excretions, secretions, meat or carcasses from infected animals. The incubation period varies from 4 up to 19 days and virus excretion can start already at this stage. Viraemia (virus presence in blood) usually begins 2-8 days post-infection (dpi) and persists for at least 3 to 5 weeks. In the acute phases of infection, the virus titre in tissues and blood is very high. This is due to the absence of fully neutralizing antibodies, and then an intermittent viraemia is usually maintained during convalescence, that can be detected up to 3 months in the recovered animals (Gallardo et al; 2015a). These animals can maintain the virus in tissues for up to 99 days or even more time as demonstrated in past studies what point out the potential for virus transmission by contact or feeding (Hess, 1981; Gallardo et al., 2015a). This is particularly important in the case of death WB in the forest, since it has been already demonstrated a cannibalism behaviour of the WB exists. An investigation carried out in the USA demonstrated virus presence in tissues after 120 days in recovered asymptomatic animals after inoculation with the Brazilian isolate, and healthy animals became infected with this material through feeding (Mebus and Dardiri, 1980).

As mentioned, ASF clinical presentation is influenced by multiple factors such as virulence of the virus strain affecting, the host and breed, the dose and the route of infection (Sánchez-Vizcaíno et al, 2015 Sánchez-Cordón et al., 2017;). This matter has been well studied in the past and again in the ASFV infections caused by the genotype II ASFV strains circulating in Eastern Europe in the wild boar and domestic pig populations since 2007 up to present (Mebus & Dardiri , 1979; Hess, 1981; Mebus et al, 1980,1981, 1983 ; Blome et al., 2013; Vlassova et al., 2015, Gallardo et al., 2018; 2019b).From these studies it is concluded that whatever the genotype is, the disease presentation and the disease evolution in the past and in the current epizooty of Europe and Asia, always exhibits a same pattern. ASF clinical forms

vary from peracute, acute, subacute, chronic and subclinical that are produced by highly virulent, moderately virulent or low virulent/attenuated isolates (Mebus, et al., 1980, 1981; 1983; Hess,1981; Pan & Hess, 1984; Gómez-Villamandos *et* al., 2013; Sánchez-Vizcaino *et* al., 2015). A good characterization of the circulating viruses will give insights about what should be expected in the field, with a better disease recognition and, therefore, early detection that will influence in the success of the control eradication programmes.

Highly virulent viruses are usually responsible for the peracute and acute forms with high mortality rates that may reach 100% within 4–9 days post-infection. In peracute ASF, affected animals can die suddenly as soon as 1–4 days after the onset of clinical signs with no so evident lesions in organs. Pigs showing the **acute forms** of the disease display mainly a febrile syndrome moderate anorexia, lethargy and weakness, huddle together and in the final stages, suffer respiratory disorders characterised by rapid laboured breathing, with nasal secretions caused by pulmonary oedema, and deaths are expected from 3-8 days post infection. Exanthemas are very evident (pinkish almost purple skin due to intense hyperaemia), and/or cyanotic foci, which appear as irregular in the skin of the extremities, ears, chest, abdomen and perineum, and also haematomas and necrotic areas, though these lesions usually are more intense in pigs infected with moderately virulent isolates. Internal lesions are mainly characterized by hyperaemic splenomegaly and haemorrhages in organs, particularly in the visceral lymph nodes, petechial haemorrhages in the kidneys, bladder mucosa, pharynx and larynx, pleura and heart, and an excess of natural fluids in body cavities and spaces (Sánchez-Vizcaino et al, 2015). Haemorrhagic discharge from the anus (melena) is sometimes observed. The distribution and frequency of these lesions are variable and most are seen in other swine diseases such as classical swine fever. Acute forms of the disease by virulent viruses have been described in all the epidemiological scenarios were the disease is currently present. (Pan and Hess, 1984; Gómez-Villamandos et al., 2013; Sánchez-Vizcaino et al., 2015).

Moderately virulent viruses lead to the appearance of **acute, subacute, chronic and subclinical** forms of the disease. The reasons why ASF exhibits this significant variety of clinical signs are not precisely clear, but surely the immune mechanisms of defence developed by the hosts play a role. Pigs may show persistent or fluctuating temperature responses for up to 20 days. Some pigs may stay in good condition, while others show similar but less severe clinical symptoms than those observed in the acute process. Mortality rates range 30–70%, the lowest rate displayed in adults. The death usually occurs after 2-3 weeks post infection. (Pan & Hess, 1984; Mebus & Dardiri, 1979; Gomez-Villamandos et al., 2013; Sanchez-Vizcaıno et al., 2015; Mur et al., 2016). Animals infected with moderately virulent isolates show a variety of clinical symptoms depending on the course of the disease. Some of them can exhibit **acute and subacute** forms, with clinical signs developing slowly, and deaths between 7 to 20 dpi. In the subacute presentation the vascular lesions, mainly haemorrhage and oedema, are more marked than in acute courses as the lesions develop fully (Gómez-Villamandos et al., 1995, 2013). Pulmonary oedema does not usually occur, though pulmonary haemorrhages does as well as foci of necrotic pneumonia. To further complicate matters, distinction should be made between the **chronic disease** that is occasionally encountered during the acute and subacute virus infections. Mortality in the chronic animals is low, affecting between 2 and 10% of all the sick animals. Chronic ASF is especially difficult to recognize, because it is extremely variable in its appearance. Clinical signs and lesions are not specific and may persist for several months with no particular signs, other than stunting or emaciation, or it may mimic a variety of illnesses. In addition to stunted growth and emaciation, some clinical signs may include skin ulcers, arthritis, and lameness due to swollen of joints, respiratory symptoms and abortion. Several symptoms are as the result of secondary bacterial infections, the most significant being articular alterations and in reproduction.

It is likely that the incursion of ASF in the Americas were by a moderate virulent virus strain. Although the initial diagnosis were made in outbreaks where mortality was high and the acute disease was prevalent, very shortly thereafter the disease was found to be widespread and considerably reduced in mortality. In all probability, the disease had been in the country for quite some time and had spread extensively. It was diagnosed only after it began to appear in the acute form with mortality high enough to cause alarm. (Hess, 1981)

In areas were moderate virulent virus has been found, the **Low virulent or attenuated viruses** have been also described, causing chronic and unapparent forms of disease. *In vivo* experiments with attenuated viruses show both patterns, chronic and subclinical, that may also be observed simultaneously in the same pen.

Virus evolution Disease presentation and recognition

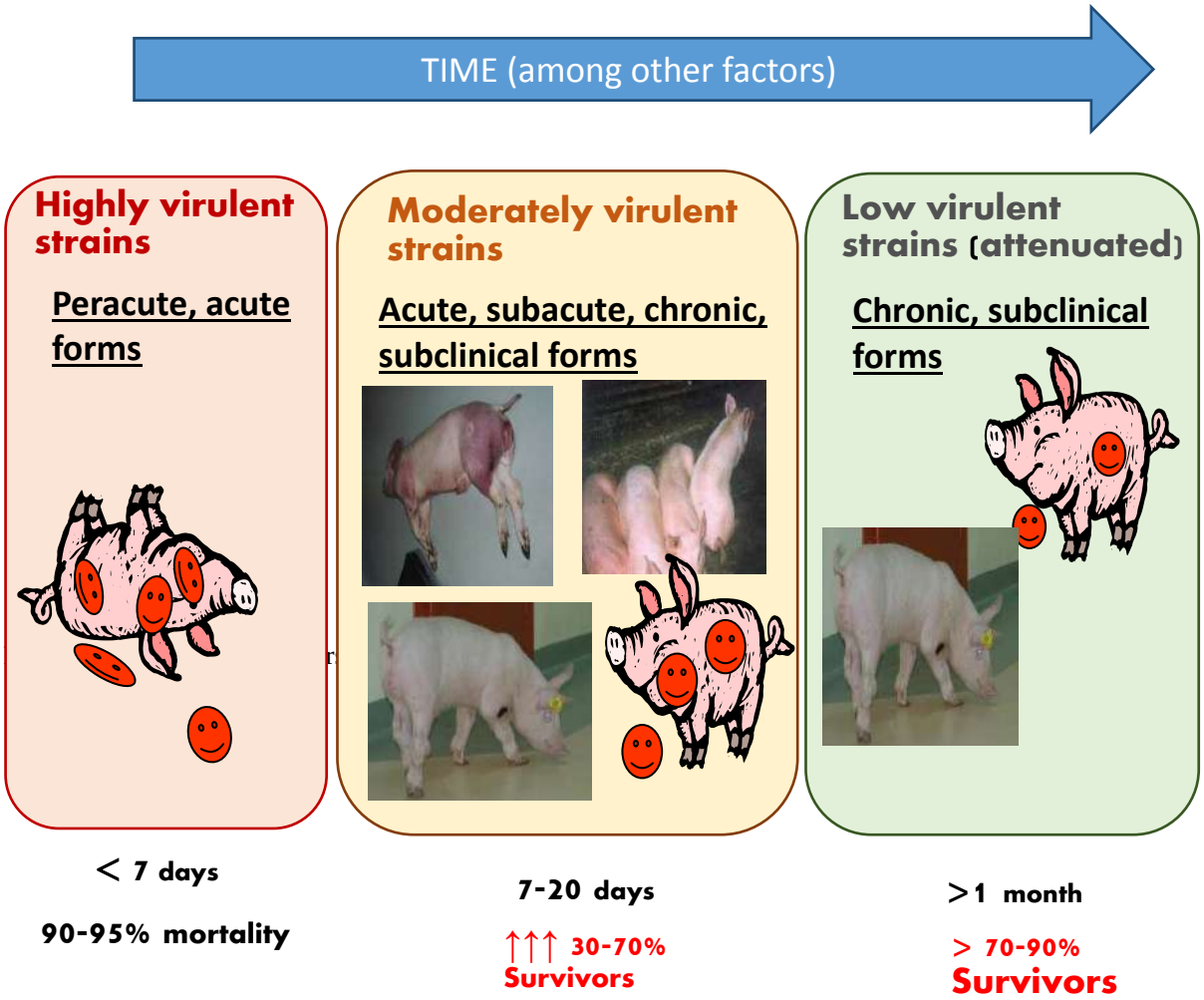
The chronic and subclinical forms of the disease are the result of appearance of moderate and low virulent/attenuated virus strains. This is the natural attenuation and virus evolution of ASFV. Chronic forms have been previously described in the Iberian Peninsula and in the Dominican Republic (Mebus & Dardiri, 1979; Leitao et al., 2001; Sánchez-Vizcaino &Arias, 2019), and it has been recorded in the *in vivo* experiments with virus strains from Europe in the recent epizooty (Gallardo et al; 2016, Nurmoja et al, 2017). Arthritis and skin ulcers have been associated mainly with the chronic disease that occurred in large numbers of pigs that received modified live virus vaccines in Portugal and Spain during the early 1960s (Sánchez-Vizcaíno, *et* al., 2015).

Subclinical, unapparent, forms are usually reported in endemic scenarios, in which clinical signs are mild or even absent. These ASF infected animals can be only identified by laboratory diagnosis. The detection of this animals is

of major importance since they may act as potential virus carriers, mainly during the recovering time and up to three months after infection. Their presence pointed out new changes in the circulating virus strains and therefore disease evolution. The detection of these animals, survivors, recovered and asymptomatic animals should not be underestimated.

It has been shown that all these clinical forms (acute, subacute, chronic and subclinical) are present in a variable percentage in regions where ASF is present after months or years. Also, it has been well established to be present in the past in the affected countries, as well as in the recent epizooty in Europe. The presence of high-virulent, moderate and low virulent virus isolates belonging to a same genotype moving simultaneously in a specific zone have been demonstrated (Gallardo et al., 2018, 2019 Nurmoja 2017). Therefore, a key point to efficiently combat the disease is to remind that regardless the *genotype*, and the virulence of the virus in the primary outbreaks, the evolution of the clinical infection after a time (several months or years) shows a common pattern: the presence of per-acute/acute, subacute, chronic and subclinical forms of the disease, in a percentage variable depending on the time and the epidemiological scenario.

Disease recognition is a key point for an efficient early detection of the disease. This recognition may vary due to a number of parameters as explained above. ASF may doesn't resemble a typical picture. Moreover, ASFV introduction in a farm is not easy to detect at the beginning, since only fever and deaths with some hemorrhagic lymph nodes will be observed in few animals. Several cycles of infection are required to arise a significant virus amount before it began to display the typical characteristics of the acute disease with high mortality of the animals in a pen. Following an initial infection in several pigs, a second wave of infection 12-14 days later should be expected, that will lead to a significant number of deaths, thus increasing the infective pressure. A third wave later and a devastating wave of deaths will take place (Hess, 1981; Arias et al., 2014; Gallardo et al., 2015). In high-risk areas, sudden deaths of few animals should not be merely attributed to common causes but, rather more be treated with the upmost seriousness. Depending on the type of production system, the biosecurity conditions, husbandry, management and organization different evolution patterns of ASFV infection could be expected, that also will influence the movement and spreading of the ASFV infection waves. One key element in prevention ASF introduction is not to subestimate the disease presentation, be alert, and not to minimize the appearance of clinical symptoms, i.e fever, even if they are affecting few animals, as well as to carry out periodical clinical checking.



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Birth weight effects on the efficiency of genetic transfer from production nucleus to commercial production level

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Introduction

Despite the wealth of information on the many factors that affect sexual maturation and the early breeding performance of gilts, it has been difficult to establish the needed protocols that produce a consistent supply of "good quality" replacement females. In the context of the latest genotypes available to the production industry, it is important to keep addressing the changing biology of the nucleus sows from which replacement gilts are generated. The phenotypes of some of the emerging sub-populations of contemporary sows prompted a discussion about how these phenotypes might affect the efficiency of replacement gilt production and the performance of replacement gilts at the commercial level of production. Therefore, as our contribution to the present Workshop, we would like to discuss the results from a recent large-scale project that provide insights into the link between birth weight phenotype (BWP) and 1) potential inefficiencies in replacement gilt production at production nucleus level, and 2) variable sow lifetime productivity (SLP) at the commercial level of production.

A key feature of these BWPs is that they need to be measured and then managed at the level of the individual gilt or nucleus sow. This is primarily because the "preferred" BWPs are not reflected in the commercial breeding value (genotype) of production nucleus sows from which replacement gilts are derived. Indeed, some of these phenotypes result from interactions among component growth and reproductive traits that cannot even be measured in the live animal. However, the resulting range of "reproductive" phenotypes produced by these interactions can have very different impacts on SLP, measured as either the number of replacement gilts produced per nucleus sow lifetime, or the lifetime production of quality weaned pigs at commercial level. By understanding the origin of these phenotypes and the underlying physiological traits involved, it is possible to propose management strategies to optimize genetic transfer in integrated production systems. In many ways, the possibility that detrimental sow phenotypes can be identified that are not reflected in the estimated breeding value ascribed, is similar to earlier discussions around relative boar fertility and impacts on the efficiency of genetic transfer using artificial insemination (Foxcroft et al., 2008).

Birth traits that determining the efficiency of replacement gilt production

The ability to predict a sow's litter birth weight phenotype (BWP) is important and has considerable ramifications for the efficiency of replacement gilt production and the lifetime productivity of gilts produced. Identifying sows that repeatedly display the low BWP would allow producers to selectively apply the relevant management interventions at farrowing that would increase early post-natal survival. In the most extreme low BWP population (bottom 15%) at production herd level, Smit et al. (2013) reported that no sow first giving birth to a low birth weight litter produced a high birth weight litter at any subsequent farrowing. Based on the concept that *"Measuring and managing sow (litter) birth weight phenotype (BWP)at production nucleus/multiplication level would improve the efficiency of replacement gilt production, and hence genetic transfer to production level"* a large scale collaborative research project was developed and involved a four-year analysis of 1) sow, litter and individual gilt BWP at the production nucleus level, and 2) impacts on the down-stream retention of gilts to the Pre-selection and Selection stages of development in a large production system in the USA. Later parts of the study not discussed here, also looked at the effect of "litter of origin" on retention and SLP of replacement gilts once bred.

When considered on the basis of individual pig birthweight, the critical cut-off for mortality in replacement gilts at 4 days of age was 1.18 kg and is similar to that reported previously. Gilts weighing less than 1.0 kg at birth had increased pre-weaning morality rates and little chance of surviving until weaning (Magnabosco et al., 2015). Those gilts that do survive past the nursery phase have poor growth until finishing and are significantly lighter than their higher birth weight littermates. Additionally, as future replacement females, low birth weights negatively impact their reproductive potential. Variation in gilt birth weight was negatively correlated to ovarian and uterine development (Deligeorgis et al., 1984), and Flowers (2015) suggested that below a minimum birth weight of 1.1 kg gilts simply do not have the reproductive machinery to be efficient reproductively, no matter how well they are managed later in life. Magnabosco et al. (2016) reported that gilts weighing less than 1.0 kg at birth, and still selected as replacements at 170 days of age, produced fewer pigs over three parities and remained in the herd for less time. Furthermore, Almeida et al.

(2014) reported that although low birthweight gilts were at risk for non-selection at breeding, there was no effect of birth weight on age at puberty.

A "low litter birth weight" carries all the same risks described above for individual low birth weight. In agreement with Smit et al. (2013), a negative relationship between total born (litter size) and litter average birth weight (average of all pigs in the litter) was reported in the current study. More importantly for the proposed approach to improving the efficiency of replacement gilt production, some 15% of sows can be identified within breeding herd populations that exhibit an extreme "low" average litter BWP over consecutive parities, irrespective of the total number of pigs born. Smit (2013) reported that sows giving birth to a low BW litter, again give birth to a low BW litter, or at best medium BW litter at the next farrowing, and that the correlation coefficient to determine the repeatability of average litter birth weight across successive parities is reasonably high (r=0.49 in later parities).

Overall, the individual BW of pigs born (n = 45,523) over 1 - 7 parities (n = 3,244 litters) were used to determine the BWP of multiplication sows (n = 644; PIC) producing Camborough replacement gilts (n=7644) (Table 1).

Across all litter sizes born, there was a negative relationship between litter size (total pigs born) and average litter birth weight (y = - 0.033x + 1.84; R^2 = 0.24; P<0.0001), representing a 600 g difference between the smallest and largest litters (Figure 1). In contrast, the variation in litter average birth weight among litters with the same total born was greater (mean = 1200 g, range 900 to 1500 g). As litter size increases, there is an increasing lack of high birth weight litters due to increased prolificacy.

Table 1. Summary statistics of measured traits of the birth sow used to determine phenotypic classifications.

	n	Mean	SD	Min	Max
Individual piglet weight (kg)	45,523	1.34	0.34	0.23	2.80
Individual litter data					
Average litter birth weight (kg)	3,244	1.37	0.23	0.68	2.54
Average total born)	3,244	14.5	3.5	1.0	25.0
Sow litter phenotype[1,2]					
Birth weight phenotype (kg)	644	1.35	0.16	0.86	1.96
Average total born within phenotype	644	14.7	1.6	10.3	21.0

[1]To calculate sow litter phenotype, only sows that had at least two consecutive litter measurements with >10 total born were used.

As shown in Figure 1, the litter average birth weights of the most prolific sows with more than 20 total pigs born are lower than the population average litter birth weight of 1.37 kg. In contrast, across the entire range of litter sizes from 10 to more than 20 pigs total born, there is a population of sows that have low birth weight litters that cannot be attributed to prolificacy in the sense of total pigs born.

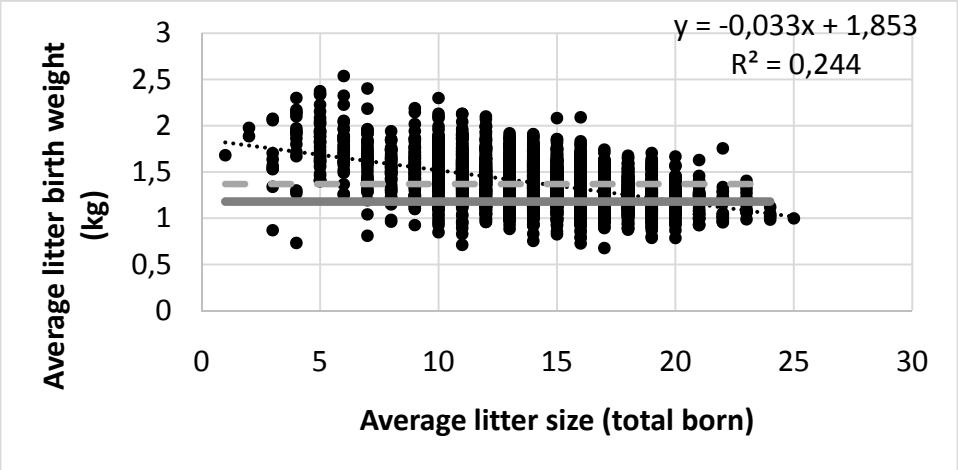

Figure 1. Relationship between litter size (as total number of pigs born) and litter average birth weight. The average litter birth weight over the population is 1.37 kg (dashed grey line). The solid grey line shows the critical breakpoint in individual birth weight (1.18 kg) for mortality at d4 after birth.

Sow BWP was determined over at least two successive parities with litters with >10 total born for sows (n = 644), with an overall mean litter birth weight of 1.35 ± 0.16 kg: these sows then produced some 7,664 replacement gilts that were individually tagged at birth. Overall, the Low (L) and High (H) BWP sows represent 13.4 and 14.6%, respectively, of overall population (Table 2).

Table 2. Summary statistics of measured traits in low (L), medium-low (ML), medium-high (MH) and high (H) birthweight phenotype sows.

	Sow Birthweight Phenotype (BWP)				
	L	ML	MH	H	P <
Sows					
n	85	250	209	100	644
% per category	13.2	38.2	32.5	15.5	
Total born	15.3 ± 0.2	15.2 ± 0.1	14.5 ± 0.1	13.6 ± 0.1	0.001
Average litter weight (kg)	1.12 ± .01	1.28 ± .01	1.42 ± .01	1.62 ± .01	0.001
Gilts individually tagged					
n	985	2882	2406	1075	
% per category	13.41%	39.22%	32.74%	14.63%	

Retention rates. A preliminary analysis to determine selection outcomes for pure-bred L3 replacement gilts within the production nucleus flow identified large variations in the proportion of gilts selected from particular litters (Figure 2). Sectors of the pie-chart show the percentage of total litters in which varying proportions of gilts born were not selected for transfer to the nucleus farm GDU. In about 10% of all litters, between 50 and 75% of gilts born (purple sector) were not selected. An even more extreme outcome was seen in 4% of the litters (light blue sector), with more than 75% of all gilts born being considered "non-select". Using existing selection criteria, a large proportion of gilts were considered "non-select" at weaning or around 140 days of age on the basis of relatively poor growth performance. Combined with the higher proportion of low birthweight pigs that die in the early post-natal period, our prediction (which was confirmed in commercial replacement gilts) is that these high proportions of dead and non-select gilts are born to sows with a repeatable low birth weight phenotype.

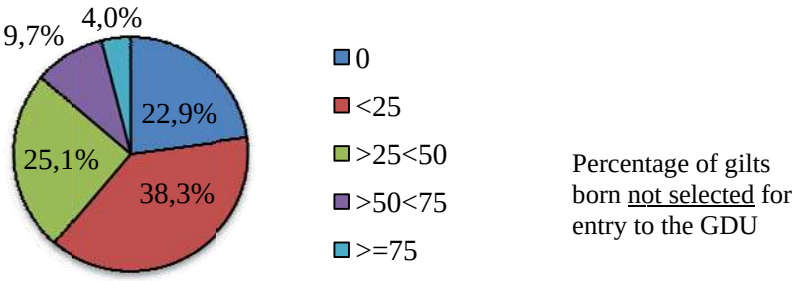

Figure 2. Preliminary analysis of selection outcomes for pure-bred replacement L3 gilts identifies large variations in the proportion of gilts selected from particular litters.

Overall, the data on birth weight phenotype collected was the critical first step in linking gilt litter of origin (sow BWP) to the efficiency of gilt replacement production, gilt survival to weaning, retention though the pre-selection stages of gilt development, and final selection in the GDU. Gilt retention rates at various stages of production classified by the different BWPs are shown in Figure 3. As would be expected, relating retention rates to individual birth weight class resulted in greater variation in the retention rates than the sow BWP. However, even when analysed on the basis of the sow's established BWP, the trends were very similar. Gilts born to sows with the low BWP have compromised retention at all stages of production compared to gilts born to sows with higher BWPs.

Low birth weight determined either on an individual pig basis, as the mean birth weight of the birth litter, or sow's average litter birth weight phenotype is negative for pre-selection rate. Significant differences in survival are already present by day 4 and at weaning, reflecting the early crushing and poorer pre-weaning survival of low birth weight pigs and litters already seen at the commercial level of production (Smit et al., 2013). Also consistent with the present results, Magnabosco et al. (2015) reported that gilts weighing less than 1.0 kg at birth have increased pre-weaning morality and little chance of surviving until weaning and those gilts that do survive past the nursery phase have poor growth until finishing and are significantly lighter than their higher birth weight littermates. As a disproportionate number of low birth weight gilts are born to sows with the low and medium-low BWP, removing 10 to 15 % of nucleus sows with the extreme low BWP would improve gilt retention rates in the remaining sows. Additionally, removal of the few very low birthweight gilts born to sows with the higher BWPs would further improve the retention of gilts through the Pre-selection and Selection stages of production. In terms of minimizing lifetime nucleus sows' costs per replacement gilt produced, an early culling strategy for sows determined to exhibit the extreme low BWP seems justified. If culling was based on litter birth weight data from the first two farrowings, even pure-bred nucleus replacement gilts could be removed as potential replacements at the Pre-selection 2 stage at around 170 days of age, at which time their birth dams would already have produced a second litter.

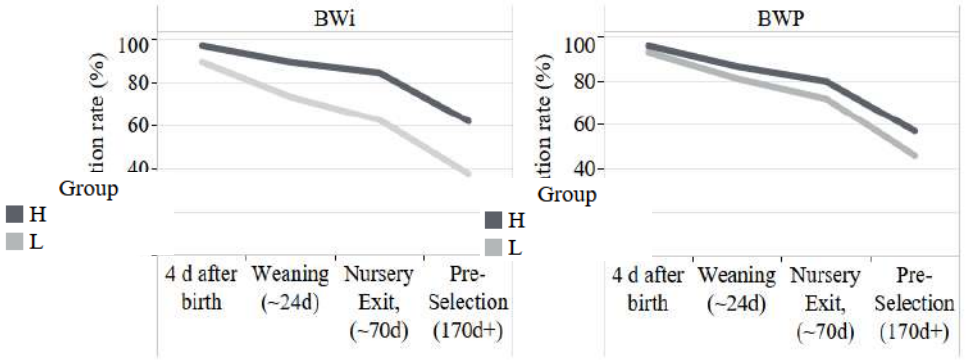

Figure 3. The effect of individual gilt birth weight (Bwi) and sow birth weight phenotype (BWP) on retention rate (%) from birth to "Pre-selection" at 170 d. Retention rates for the extrem High (H) and Low (L) phenotypes are shown.

From a genetic transfer perspective, the production of very few replacement gilts in the productive lifetime of some sows in the production nucleus/multiplication herd, represents a poor genetic investment. These sows only "earn" their genetic premium if their genetic potential is effectively passed on to the Camborough replacement gilts used for terminal line production. The disconnect between a high genetic merit for genetic traits included in the estimation of EBV and the production of few replacement gilts carrying these genes to the level of terminal line production, is analogous to the problem of high EBV boars that are determined to have relatively low fertility when used for AI, either in single-sire matings, or when used in pooled semen doses out of which the poorer boars sire very few progeny. In the larger production enterprise that manage their own boars' studs and production nucleus/multiplication farms, the opportunity to optimize genetic transfer down to the level of terminal line production offers significant economic benefits. In both cases, the additional replacement costs of early culling of unproductive boars and sows is very largely offset by the enhanced performance of the boars and nucleus sows remaining in production.

Growth performance. The associations among the different birth weight classifications and gilt weights at Pre-Selection at 170 days of age are shown in Figure 4. Determined on an individual pig and birth litter weight basis, birth weight had a highly significant effect on weights at 170 days and comparable trends were seen when sow BWP was used in these comparisons. The first and expected conclusion is that birth weight reflects post-natal growth potential, again reflecting results from studies at the terminal-line level of production (Smit et al., 2013) and in comparable replacement gilt studies (Amaral Filho et al., 2010, Magnabosco et al., 2015).

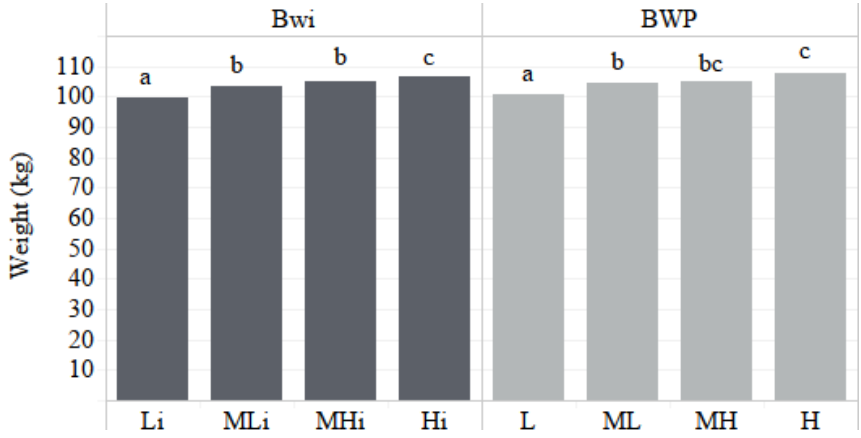

Figure 4. The effect of individual birth weight (Bwi) and sow birth weight phenotype (BWP) on gilt weight at Pre-selection at 170 days of age. [a.b,c.d] Differences between columns within birth weight category are significantly different ($P < 0.05$).

However, in all the birth weight classifications, gilts had adequate growth for achieving sexual maturity in response to stimulation with boars at the final Selection stage of gilt development. The study of Beltranena et al. (1991) defined a threshold growth rate to puberty of 0.55 kg/day below which the attainment of puberty was delayed. As with most recent surveys of gilt growth performance in systems that use feeding to appetite throughout gilt development, the data in Figure 4 suggest that even the low birth weight gilts that are retained to the Pre-select 2 stage of development have sufficient growth performance to express their inherent sexual precocity. Therefore, little relationship would be

expected between growth rate classification and the measured responses to stimulation with boars in the GDU/BEAR facilities. If the latter assumption is true, and the post-breeding performance of low BWP progeny is not compromised, this raises questions about existing selection strategies that do not favor the retention of gilts with relatively low growth performance amongst their birth cohort. However, taking into account the cost of feed, non-productive days, born alive and litter uniformity, starting at breeding, Amaral Filha et al. (2010) recommended that gilts should achieve a growth rates between 600 and 770 g/d for optimal performance. Again, most gilts at the Pre-select stage in the present study would meet these growth requirements.

At the other end of the growth rate spectrum, the excellent growth performance of gilts born to high BWP sows may be an increasing problem for the industry, as individual gilts are achieving growth rates to puberty of > 0.7 kg/day. As there is no expected link between high growth rates and age at first estrus, some later maturing gilts achieve weights at HNS and at breeding that are above industry benchmarks. If the efficiency of inducing pubertal estrus is low, or the pre-stimulation management or health status of replacement gilts at the time of final selection is poor, over-weight gilts at breeding becomes a major risk factor for gilt retention in the breeding herd (Amaral Filha et al., 2010).

Responses to puberty induction in the GDU: The associations among birth weight classifications and responses to boar stimuli in the purpose built GDU/BEAR facilities used in the trial are shown in Figure 5. Collectively, the data confirms that for those gilts retained beyond the Pre-select 2 stage and moved to the GDU for stimulation with boars, there was little relationship between individual gilt birth weight or sow BWP and age at puberty, having commenced stimulation with boars at around 182 days of age. However, because birth weight was positively associated with growth rate, there was also a positive association with weight at pubertal estrus, approaching a 10 kg difference in pubertal weight between gilts born to the low and high BWP sows (Figure 6).

The 80-83% response rate of gilts with a recorded first estrus (HNS) puberty within 35 d of initial boar exposure in the BEAR systems indicates effective use of the stimulation protocols. Although the on-farm GDU/BEAR systems can lapse into an almost continuous-flow style of management, early identification of a pubertal heat and early culling decisions for non-select gilts are essential if a gradual increase in entry-to-service intervals and overcrowding of GDU pens are to be avoided. As with the off-site GDU/BEAR sites that were part of this study, management of all GDUs on an all-in/all-out basis is important for maintaining breeding herd efficiency.

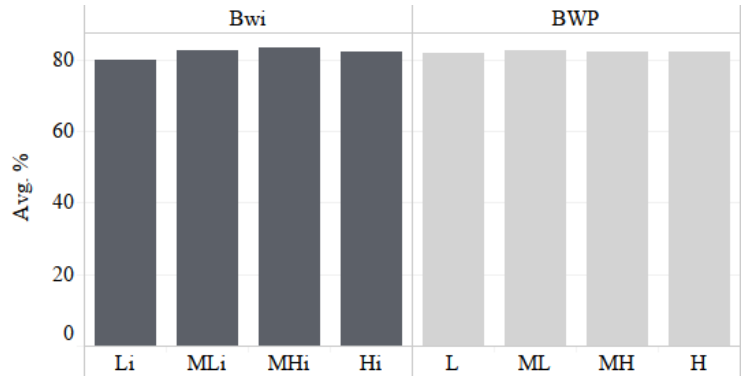

Figure 5. The effect of individual birth weight (Bwi) and sow birth weight phenotype (BWP) on the percentage of gilts with a recorded heat-no-serve within 35d of boar exposure.

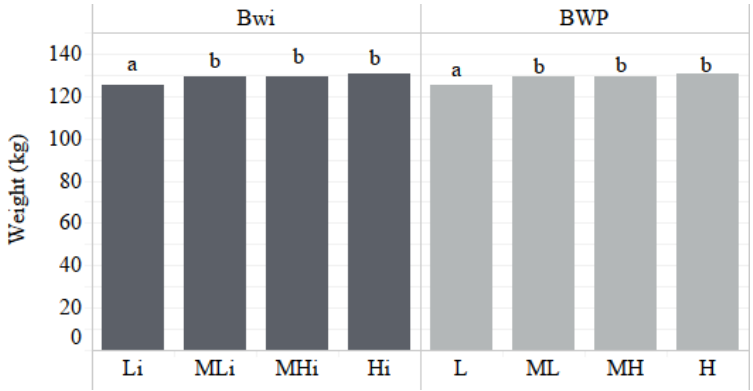

Figure 6. The effect of individual birth weight (Bwi) and sow birth weight phenotype (BWP) on weight at Selection after starting the selection program at around 180 days of age. [a.b,c] Differences between columns within category are significantly different ($P < 0.05$).

Conclusions

Poor survival to weaning, and lower gilt retention rates during development, are critical issues for low birth weight gilts and for gilts born to sows with a low BWP. Retaining sows in the production genetic nucleus population if they exhibit a repeatable low BWP negatively impacts the efficiency of replacement gilt production and represents a poor return on the investment in their high genetic merit. Nucleus sow culling strategies aimed at the early removal of the 10 to 15% of sows with the extreme low BWP, as well as non-selection at birth of the lower birth weight gilts from other litters, will improve the overall efficiency of the nucleus/multiplication farm in terms of efficient genetic transfer.

For pre-select gilts entering the GDU stage of production using current pre-selection criteria, lifetime growth performance and litter of origin traits have little impact on breeding performance. However, gilts born to the higher BWP sows were heavier at breeding and their retention in the breeding herd would be considered as a risk factor for SLP. The risk of gilts being too heavy at breeding, with negative impacts on subsequent retention in the breeding herd, emphasizes the importance of efficient GDU/BEAR programs that minimize entry to service intervals. The present study demonstrated that such programs can be implemented in large commercial systems and the success of these selection and pre-breeding programs makes a fundamental contribution to achieving acceptable SLP.

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Colostrum and milk intake and composition, and effects on postnatal gastrointestinal tract development and production until weaning

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Introduction

The growth, development and functionality of the gastrointestinal tract (GIT) is a dynamic and evolving process that prepares the young pig, both before and after parturition, for its future growth, development and ultimately, survival. The perinatal development of the GIT can be divided into three phases: the *prenatal phase*, characterized by minimal stimulation from the GIT lumen; the *neonatal phase*, associated with changes caused predominately by colostrum and milk intake; and the *post-weaning phase*, associated with marked changes to, and the adaptation of, the GIT to solid feed (Pluske, 2016). This review focusses only on the *neonatal phase*.

Discussion of the impacts of colostrum and milk composition on post-natal gastrointestinal tract development and production is highly relevant to the worldwide pork industry mainly because there has been a steady increase in litter size in the last years, commensurate at times with little or no change in pre-weaning survival. Between 1990 to 2010, litter size on average increased from 11 to 14 piglets born per sow per litter with some countries reaching an average more than 16 piglets. In hyperprolific sow lines, it is not uncommon to have litters up to 18–20 piglets, or more, born alive (summarised in Oliviero et al., 2019). This increased number of piglets has become a major challenge for both sow physiology during pregnancy, at parturition and during lactation, as well as for postnatal growth and survival. Larger litters have a direct impact on the piglets at birth, with the higher the number of piglets born in a litter resulting in both a lower piglet birthweight and a higher variation in piglet birthweight within the litter (summarised in Oliviero et al., 2019). A greater number of piglets born than the available teats at the sow's udder, a lower birthweight and a greater birthweight variation all increase the piglets' competition for colostrum intake (Declerck et al., 2017). A reduced colostrum intake in the first 24 hours of life has negative effects on piglet survival (Devillers et al., 2011), especially for piglets of low birth weight and low weight gain, and hence is one of the major causes of neonatal mortality. Similarly, lower birthweight and long farrowing duration are associated with lower piglet vitality at birth, which can delay the access to the udder (e.g., Islas-Fabila et al., 2018). Furthermore, and in larger litters, an increased number of piglets born with signs of intrauterine growth retardation is also described (e.g., Matheson et al., 2018).

Newborn piglets are born with functionally mature innate immunity, but without the protection of immunoglobulins because of the epitheliochorial nature of the placenta and with polarised Th2-type immunity. The Th2-type immunity is mediated by high levels of progesterone and Th2 cytokines produced in the maternal–foetal interface (Oliviero et al., 2019). Neonate piglets must therefore procure maternal immunoglobulins from ingested colostrum for passive immune protection, before they start to adequately produce their own immunoglobulins at approximately 3–4 weeks of age (Rooke & Bland, 2002).

Colostrum and milk composition

Colostrum is obviously an important secretion of the sow and hence its composition typically reflects the physiological biology of the sow at the time, and the evolutionary requirements of her progeny (Pluske et al., 1995). As described originally by Klobasa et al. (1987), and seemingly with little major changes since this time (Theil & Hurley, 2016; Zhang et al., 2018), colostrum total solids and protein contents in the first 6 hours of lactation were higher, while fat and lactose contents were lower, than in mature milk. Decreased total protein and whey protein contents and the parallel increased fat and lactose contents, with a nearly unchanged total solids level, indicates the compositional changes associated with the transition from colostrum to mature milk. The high protein content of colostrum is largely due to immunoglobulin (Ig), and indeed and during the first 6 hours of life, IgG accounts for nearly all of the protein in colostrum but plays a decreasing role in sow milk as lactation proceeds. After 2 weeks of life, IgA levels begin to increase and at the end of lactation, IgA constitutes ~40% of the total whey protein (Figure 1).

More recently, Picone et al. (2018) used a ^{1}H–NMR-based metabolomics approach to explore colostrum compounds with a maximum 10 kDa molecular weight in three pig breeds: Large White, Landrace and Duroc, respectively. Colostrum sampling was performed during parturition, after the birth of the first piglet and before the parturition of the last, and across all teats. A total of 25 metabolites were identified, representing monosaccharides, disaccharides (such as lactose), organic acids (lactate, citrate, acetate and formate), nitrogenous organic acids (such as creatine) and other compounds including nucleotides. Principal Components Analysis showed clustering due to breed and seasonal effects, with lactose being the main compound determining the assignment of samples into different clusters according to sow breed. Among the identified metabolites, acetate and taurine showed positive effects on

piglets' performances from birth to day 3 of age and on piglets' survival rate, while dimethylamine and cis-aconitate exerted a negative influence on the new-borns' capacity to survive. Nevertheless, a preparation step for the analysis eliminated immunoglobulins and other proteins with high molecular weight, hence the absence of their well-known effects in this particular study.

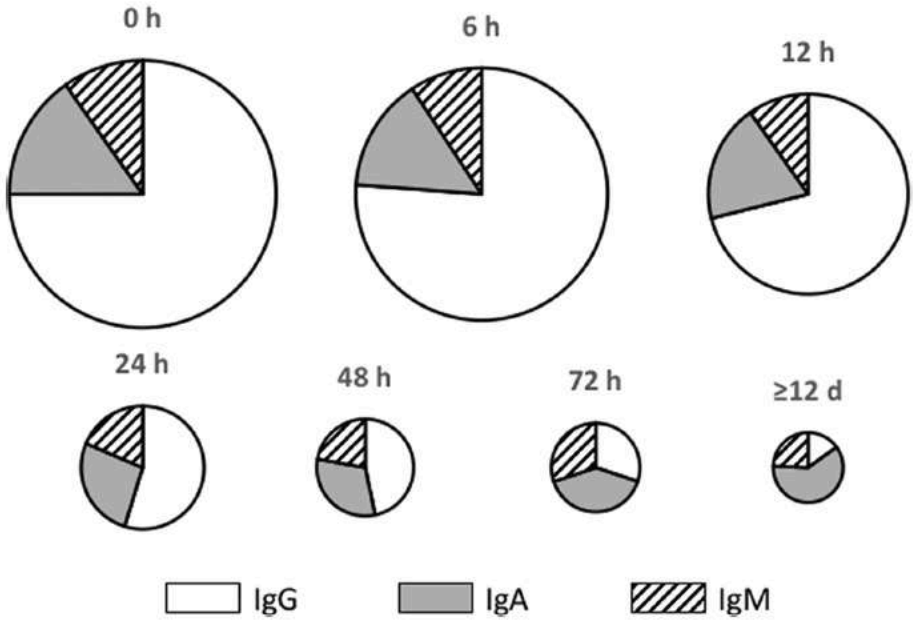

Figure 1. Relative total amounts and proportions of Ig G, A, and M in sow mammary secretions during lactation. Circles for 6 hours through ≥12 days represent the total Ig content in mammary secretions compared with the content at 0 hours (after Hurley, 2015).

Traditionally, porcine milk has been considered to be mainly composed of carbohydrates, lipids, and proteins (immunoglobulins, caseins and whey) with small proportions of minerals, vitamins, leukocytes and somatic cells. Proteins in sow mammary secretions include those associated with the milk fat membranes, caseins, mammary-derived whey proteins, immunoglobulins, hormones and growth factors, enzymes, and a wide range of other proteins. Concentrations of most milk-specific proteins typically are lower in colostrum than in milk, while concentrations of immunoglobulins and other bioactive proteins often are enriched in colostrum compared with mature milk (Theil & Hurley, 2016). In this regard, the plethora of (largely uncharacterised) compounds such as growth factors, hormones, cells, exosomes, oligosaccharides and antimicrobial factors including lactoferrin, lysozyme, lactoperoxidase, and cytokines (e.g., IL-1β, IL-6, TNF-α, IFN-γ and IL-1ra), which in one way or another are involved in the stimulation and regulation of the growth and development of the GIT after birth (Pluske, 2016), represents an area of research that is currently largely overlooked. These antimicrobial and immunomodulatory components of porcine milk have been suggested to compensate for the immature neonatal immune systems and to mitigate infectious pathogens (Zhang et al., 2018). Interestingly, milk macronutrient components do not seem to have significantly increased with enhanced reproductive performance in sows as current concentrations of protein, fat and lactose in colostrum are similar to those of 30 years ago, i.e., approximately 16% protein, 3% lactose and 5% fat (Zhang et al., 2018).

The importance of colostrum and milk intake

Not surprisingly, therefore, the growth, development, immunity and biological functions of the GIT in the postnatal period are determined predominately by the amount of the colostrum and milk the piglet consumes, as well as its composition (Le Dividich & Herpin, 2005; Devillers et al., 2011; Figure 2). Also, the importance of initial postnatal microbial colonization of the GIT to its subsequent structure and function must also be recognized (e.g., Chen et al., 2018; Wang et al., 2019). Collectively, it is of critical importance that piglets consume colostrum as quickly as possible after birth. The ability of the epithelium to take up macromolecules, including immunoglobulins, and to then transport these molecules into the circulation concludes within the first 48 hours after parturition (Sangild, 2003). Failure by the piglet to consume and absorb sufficient quantities of immunoglobulins impacts negatively not only on viability, vitality and pre-weaning survival, but also on future growth and disease.

Colostrum also supplies energy in the form of macronutrients (as lactose, fat and proteins), and during the first days of life, it's essential that the piglet consumes sufficient energy intake because the piglets' energy body reserves, especially fat, are low at birth (Theil et al., 2014). Low energy intake coupled with an inability to thermoregulate can

cause morbidity and death (Pluske et al., 1995; Theil et al., 2014). Colostrum intake consequently plays a major role in providing energy for thermoregulation.

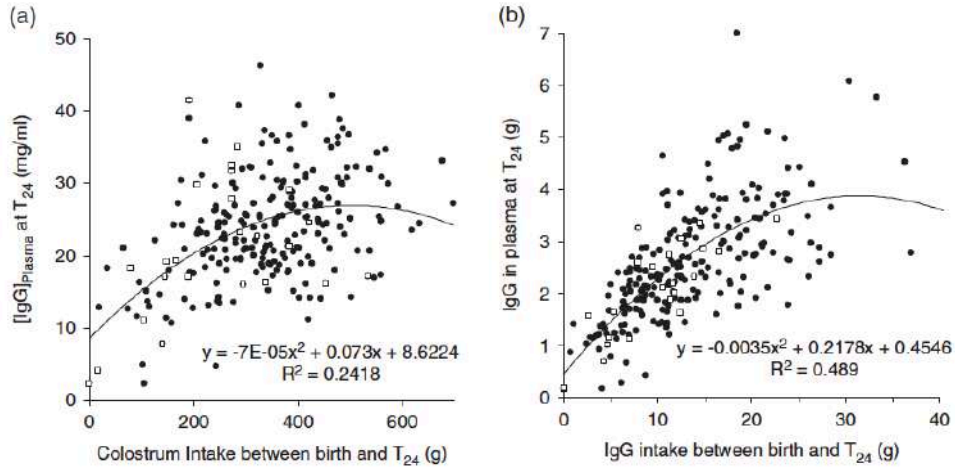

Figure 2. Relationships (a) between immunoglobulin G (IgG) concentration in piglet plasma 24 h after the onset of farrowing (T_{24}) and estimated colostrum intake, and (b) between total amount of IgG in piglet plasma at T_{24} and estimated IgG intake. Black circles represent piglets that were still alive at weaning, and white squares represent piglets that died between T_{24} and weaning (after Devillers et al., 2011).

The intake of colostrum, and subsequently milk, causes rapid organ and body growth. For example, and in a neonatal piglet weighing on average 1.45 kg at birth, small intestinal and pancreas weights contribute approximately 3.1% and 0.14%, respectively, of the total body weight (Table 1). However, and within the first four weeks after birth the weight of the piglet increases more than five-fold, with the GIT organs growing faster than many other organs (Zabielski et al., 2008). Widdowson and Crabb (1976) showed the magnitude of changes to body weight, GIT weight, size and DNA content, that occur in newborn piglets during the first day of postnatal life, compared to piglets only offered water. Zabielski et al. (2008) described these changes being associated with increased local blood flow to the GIT with a reduction in basal vascular resistance, accumulation of colostral proteins in enterocytes, and changes in epithelial cell turnover, specifically increased mitosis and inhibition of apoptosis. Numerous subsequent studies have shown that the stomach, small and large intestine, liver and pancreas grow disproportionately faster than the rest of the body (*positive allometric* growth) during the early postnatal period. For example, the stomach gains 26-28% in weight during the first day compared with a 7-8% increase in body weight during the same period (summarized in Cranwell, 1995). There is also a profound functional maturation of the stomach during the early postnatal period (Cranwell, 1995).

The major driver of piglet growth in established lactation is milk intake (Pluske et al., 1995). Estimates of milk consumption by piglets vary considerably, but in general, each piglet consumes from ~ 40-60 grams of milk at each suckle (Pluske and Williams, 1996), and there is evidence that piglets sucking from modern-day first-litter sows will drink, on average, between ~ 45 grams of milk per day (Hojgaard et al., unpublished data). However, milk production of sows differs between weeks of lactation and between herds and depends on many factors including the proportion of unsuccessful sucklings, stage of lactation, parity, litter size, voluntary food intake during lactation, body composition of the sow at farrowing, genotype, and environmental conditions and health.

Table 1. The contribution of the selected organs to the total body weight (BW) in Landrace x Pietrain crossbred piglets (from Zabielski et al., 2008).

	Suckling neonates (d 0)	Suckling piglets (d 28)
BW (kg)	1.45 ± 0.22[1]	8.17 ± 1.22
Small intestine (% of BW)	3.10	4.03
Pancreas (% of BW)	0.14	0.15
Stomach (% of BW)	0.48	0.49
Heart (% of BW)	0.76	0.56
Brain (% of BW)	2.07	0.59

[1]Values are mean ± SD.

Sow milk dry matter is converted to piglet live-weight gain at a ratio of 0.75-0.80:1, or approximately 4 g of milk per gram of liveweight gain (summarised by Pluske et al., 1995). This does not seem to have changed significantly over the last ~ 40 years (Hojgaard et al., unpublished data). In energetic terms, Hodge (1974) calculated that, in

artificially-reared pigs fed cows' whole-milk powder (reconstituted to 20% dry matter (DM)) between 10 and 30 days of age, 50 to 57% of the metabolizable energy (ME) intake was recovered in the empty body dependent upon feeding level, with more energy being retained at *ad libitum* intake. In sucking pigs slaughtered at 21 days of age, Noblet and Etienne (1987) found that between 52 and 57% of ME intake and 85% of the nitrogen supplied in sows' milk were recovered in the empty body. Furthermore, they determined that the daily rates of deposition of protein and fat were linearly related to daily empty bodyweight gain during suckling, with the deposition of 1 g of protein or fat being associated with 5.2 or 1.2 g of weight gain, respectively. An interesting feature of this work was the relatively low value obtained for the respiratory quotient (0.84). These authors commented that this value was most probably related to the high percentage of fat contained in sow's milk and to the large proportion of milk fat catabolized for energy purposes (only 54% of fat ingested was retained in the empty body at weaning; Table 2).

Nevertheless, many experiments conducted with *ad libitum*-fed, artificially-reared piglets weaned shortly after birth show that, compared to sucking piglets, offering milk liquid diets increases growth rate (summarised in Pluske et al., 1995). Presently, the growth potential of pre-weaned piglets only be fully realised when they are offered a liquid diet, because the young pig is physically unable to achieve as high a dry matter intake for dry food as when offered a liquid diet.

There are two major reasons why piglets sucking the sow are restricted in their growth rate. First, and unsurprisingly, sow milk production limits the growth of the pig. Harrell et al. (1993) calculated that milk production becomes limiting to the sucking piglet at around 8-10 days of age and that the difference between need and supply progressively increases as lactation proceeds (Figure 3). These authors estimated that, at day 21 of lactation, the sow needs to produce in excess of 18 kg per day of milk in order to supply piglets with enough energy to grow at rates comparable to artificially-reared piglets of the same age. Modern-day sows still suffer from an inability to produce enough milk and are expected to suckle more piglets also, placing additional demands on their metabolism and subsequent rebreeding performance.

Table 2. Energy, nitrogen and fat balance of suckling piglets between birth and weaning at 22 days of age (n = 20; results correspond to a litter and are expressed per piglet per day) (from Noblet and Etienne, 1987).

Item	Mean
BW[1], birth (kg)	1.39
BW gain, g/day	195
Nitrogen intake from milk, g	5.43
Nitrogen retained[2], g	4.78
Efficiency of nitrogen retention, %	88
Fat intake from milk, g	53.5
Fat retained[2], g	28.7
Efficiency of fat retention, %	53.6
Energy intake as milk, kJ	3,628
Retained energy RQ[3], kJ	1,908
Retained energy ST[2], kJ	1,984
RQ[3]	0.84
Efficiency of milk energy retention RQ, %	52.5
Efficiency of milk energy retention ST, %	54.7

[1] BW: bodyweight. [2]As measured by the Comparative Slaughter technique (ST); [3]Respiratory Quotient: retained energy estimated as difference between ME intake as milk (milk energy x 0.95) and heat production (Brouwer, 1965).

Second, the potential for lean tissue growth is restricted by the composition of sows' milk. The newborn piglet is small in body size and has a large surface area to body weight ratio relative to other farm animals and is born with small amounts of body lipid [1-2% of total body weight (Mellor & Cockburn, 1986)]. Hence, liver glycogen stores and protein in its skeletal muscle represent the main energy stores to maintain body temperature. Sows' milk is well designed for the survival of piglets because it is high in fat and it is delivered at frequent intervals (once each hour) by the sow. Sows' milk is also low in protein and, because of this relatively low protein to energy ratio (9.2 to 10.4 g MJ-1 GE), it encourages the piglet to deposit body fat. A higher dietary protein intake reduces body fat deposition (Williams, 1976; Figure 4). Sows' milk, therefore, is designed primarily to promote the deposition of fat in the piglet rather than lean tissue, and this seems to be the case for at least the first three weeks of life (Pluske et al., 1995). This phenomenon does not seem to have changed over the last 45 years (Hojgaard et al., unpublished data), and whilst early fat deposition aids the survival of piglets reared outdoors or those reared in suboptimal indoor conditions, it is doubtful whether

piglets need to deposit fat so early in life when reared in modern commercial facilities where the physical and microbiological environment are more closely controlled (Pluske et al., 1995).

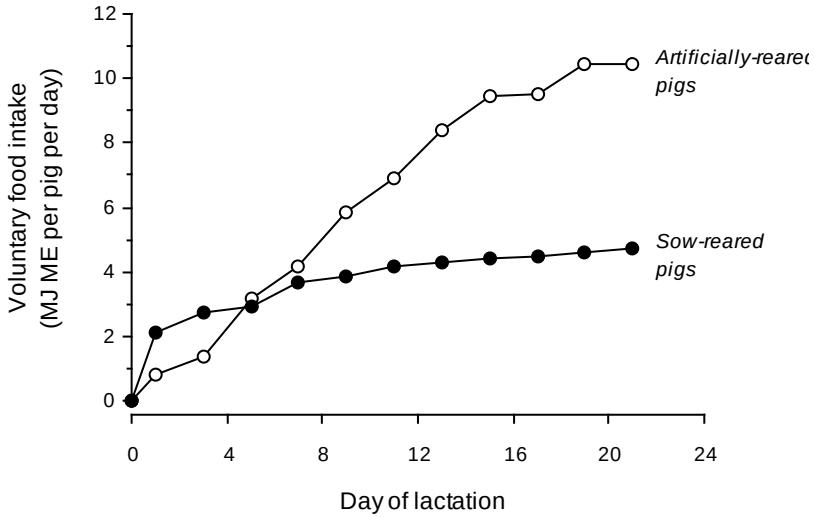

Figure 3. Voluntary food intake (MJ ME pig-1 day-1) of pigs either suckled by the sow (●——●) or fed milk replacer (○——○) following weaning at 2-3 days of age (redrawn from Harrell et al., 1993).

Be this as it may, the sow-suckled pigs (Figure 4) contained twice as much body fat as the leanest of the pigs which were artificially reared (0.46 *v*. 0.92 kg). This difference of 0.5 kg of body fat at 6.4 kg live weight might be quantitatively very important at weaning because, relative to the size of the piglet, it represents a large supply of energy which might help it adjust to the post-weaning milieu that occurs. Extra fat laid down during suckling becomes progressively less important as the pig grows because it is likely to have little effect other than to add to the total fat stores in the body. An extra 0.5 kg of fat, therefore, makes a small contribution to the fat stores of pigs at conventional slaughter weights of 80 to 120 kg (Pluske et al., 1995).

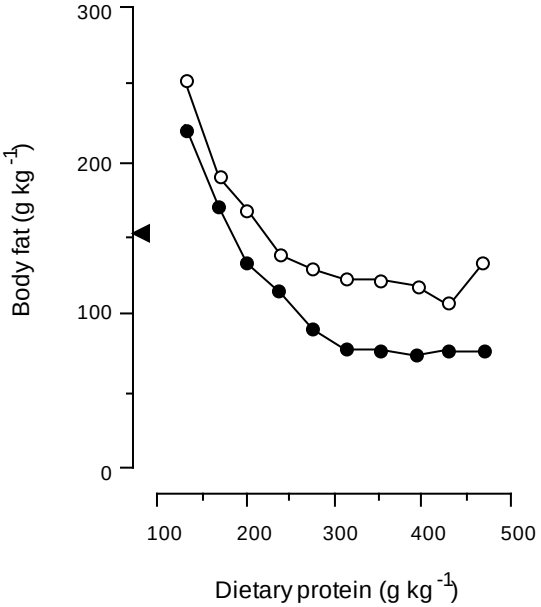

Figure 4. The effect of dietary protein on the body fat of piglets reared artificially on either 1.3 (●——●) or 2.1 (○——○) MJ of gross energy per kg live weight$^{0.75}$ per day from 1.8 to 6.4 kg live weight. The amount of body fat contained in sow-suckled piglets killed at 6.4 kg live weight is represented by (◀) and corresponds to a protein content in sow's milk of ≈ 200 g kg-1 (redrawn from Williams, 1976).

Conclusions

Organ maturation in the piglet occurs rapidly in the perinatal period in association with the transition from placental to enteral nutrition, with the enteral intake of nutrients from colostrum and milk in newborn piglets causing profound and significant structural and functional changes in the GIT. The importance of colostrum composition and colostrum intake by the piglet cannot be understated given its critical roles in the subsequent growth and maturation of the GIT. Early postnatal growth and development of the GIT is coincidental with changes in the development of the intestinal immune system, being necessary for subsequent growth, performance and survival.

Colostrum and milk perform, therefore, the following major functions: (i) provide the young pig with a source of nourishment ideally adapted to its digestive and metabolic requirements; (ii) provide the young pig with protection against microbes via the intake of immunoglobulins contained in both colostrum and milk; (iii) suppress inflammatory reactions in the GIT of the piglet; (iv) supplement the digestive enzymes of the young pig; (v) stimulate cell division and differentiation in the GIT; (vi) exercise a degree of control over metabolism; (vii) modulate the endocrine system; and (viii) contain biologically-active compounds that have the potential to influence the behaviour of the young pig. Such a suite of functions ensures piglets are provided with good sources of nutrition. However, given that the composition of colostrum and milk has changed little over time, coupled to the characteristics of the modern-day, hyperprolific sows giving birth and rearing significantly more piglets than in the past, then research into these fields is required to continue.

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From follicle pool to piglet birth weight (variation)

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Introduction

As the economic success of sow husbandry relies very much, but not solely, on the ability of sows to produce a high number of piglets per sow per year, a high sow litter size contributes to this goal. Genetic selection for higher litter size has resulted in a steady increase in sow litter size in the last decades, and this increase in litter size still continues. For example, in the Netherlands, sow litter size (total number born) has increased from 11.6 in 1996 to 13.3 in 2006 and 15.8 in 2016 (Agrovision, 2016). Concomitantly with this increase in total number born of about 0.2 piglets per year, however, also the number of still born piglets has increased (from 0.7 to 1.2 piglets) and so did the percentage of piglets that died during lactation (from 11.5% to 14.3%). Similar trends are seen in Denmark between 1996 and 2011, where litter size increased from 11.2 to 14.8, but not in the UK where live born litter size only increased by 0.6 piglet to 11.4 in those 16 years (Rutherford et al., 2013).

These higher litter sizes have consequences for both sow and piglet physiology, welfare and performance, as has been extensively reviewed (Rutherford et al., 2013, Baxter et al., 2013). In this paper, we will focus on the relations between litter size and piglet birth weight and birth weight variation, as these are indicative for subsequent piglet performance. We discuss the potential physiological background of these relations and incorporate findings of recent PhD studies on this topic at Wageningen University (Da Silva, 2018, Costermans, 2020). Finally, we discuss some breeding and management conditions affecting these litter characteristics (reviewed in Kemp et al., 2018).

Litter characteristics

Larger litters have on average lower piglet birth weights and more variation in piglet birth weight within litters (Quesnel et al., 2008). For example, in organic sows with an average litter size of 17.4±0.3 piglets, Wientjes et al., (2012b) found that each extra piglet in the litter was related with a 40 g lower average piglet birth weight, a 0.75% increase in the variation coefficient in birth weights within a litter and a 1.5 % increase in the percentage of piglets with a birth weight of less than 800 g. These relations between litter size and litter characteristics also have a genetic background, as negative genetic correlations are found between litter size and birth weight (-0.30 to -0.49), and positive genetic correlations between litter size and variation in birth weight (0.21 to 0.25) and pre-weaning mortality (0.25 to 0.45) (reviewed by Da Silva, 2018).

This lower average piglet birth weight and higher variation in piglet birth weights are relevant because of the increased chances of mortality during lactation of the lower birth weight piglets. This increased mortality is related with impaired energy reserves and thermoregulatory capacity, delayed and reduced colostrum intake and a disadvantage in competing with heavier littermates at the udder (Milligan et al., 2002, Damgaard et al., 2003, Quesnel et al., 2012). Moreover, lower average birth weight negatively affects growth performance and carcass quality of the piglets that survive (Beaulieu et al., 2010, Rehfeldt et al., 2008).

So, what causes the association between litter size and piglet birth weight (variation)?

Physiological background

Ovulation rate

Litter size is determined by underlying physiological processes like ovulation rate, oocyte fertilisation rate and embryo and foetal survival and development. Ovulation rates (as found in sow and gilt experiments all over the world in the last 36 years, reviewed by Da Silva (2018), have increased by approximately 0.2 ovulations per year, in sows as well as in gilts (see Figure 1). The variation in ovulation rate between sows can be quite considerable, e.g. varying from 17 to 49 in a group of 91 multiparous sows (Da Silva, 2018) (see Figure 2). Thus, higher litter sizes have come with high average ovulation rate, but also with extremely high ovulation rates in some sows.

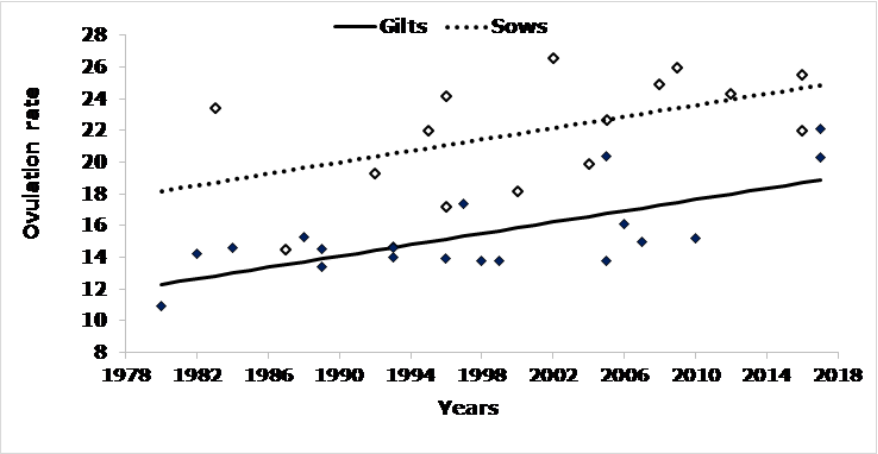

Figure 1. Ovulation rate as found in studies during the last 35 years, showing an increase of 0.2 ovulations per year in both gilts and sows (Da Silva, 2018).

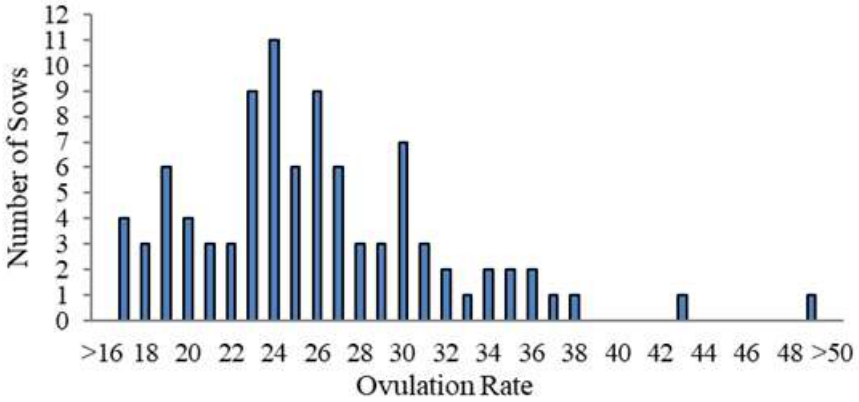

Figure 2. Variation in ovulation rate in multiparous sows (based on Da Silva, 2018).

Pre-natal survival and development

When sows are inseminated at the right time with good quality semen, fertilisation rate is considered to be 90-100% (reviewed by Kemp and Soede, 1997). A higher ovulation rate results in more implanting embryos. Each embryo needs a certain amount of uterine space to develop a placenta of sufficient length to sustain its development, growth and survival. Insufficient uterine space will result in increased embryonic and foetal mortality, and will also limit foetal development, a phenomenon that is called uterine crowding (Foxcroft, et al., 2014). This phenomenon has been eloquently demonstrated in an experiment (Père et al., 1997) in which surgical Unilateral-Ovary-Hysterectomy (UHOX) was performed to decrease uterine space per embryo (thus inducing higher levels of uterine crowding), while at the same time in another group of sows surgical ligation of one oviduct was performed to increase uterine space per embryo (thus decreasing the level of uterine crowding). As a result, the ovulation rate per uterine horn varied from 4.3 (ligation) to 8.7 (control) and 17.0 (UHOX), corresponding to the ranges of ovulation rate per uterine horn (Figure 2). These differences in ovulation numbers corresponded to differences in pre-natal survival at day 112 of pregnancy, prenatal weight and placental weight as expected in low vs. high crowding situations (Père et al., 1997).
That current high ovulation rates indeed affect embryo survival and embryo quality as has recently been corroborated by Da Silva et al. (2016) and Da Silva et al. (2017a) for sows and gilts, respectively. Figure 3 shows the increased gap between ovulation rate and number of embryos at day 35 of pregnancy, in both sows and gilts exhibiting a high ovulation rate. This gap is due to increased levels of early mortality (the gap between the number of ovulated follicles and total number of embryos) and late embryo mortality (the number of non-vital and degenerated embryos). Moreover, in gilts (Da Silva et al., 2017a) high ovulation rates were related with higher within litter variation in birth weight (ß=0.01). In sows, high ovulation rates were related with lower placental lengths, where each additional corpus luteum (CL) represented a decrease in placental length of 0.38 cm at day 35 of pregnancy. This decrease in placental length was, not surprisingly, associated with increased late embryo mortality (Da Silva et al., 2016).

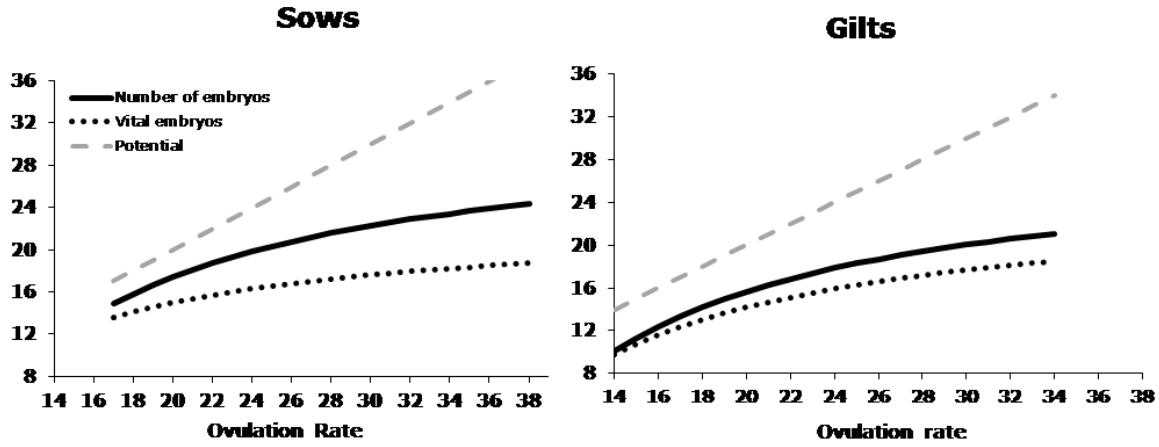

Figure 3. Relation between ovulation rate and the predicted number of total (thick line) and vital (this line) embryos at day 35 of pregnancy in sows and gilts. The dashed line (- - -) represents the potential number of embryos (i.e. ovulation rate) (Da Silva, 2017). Note: The difference between total and vital embryos is considered to be late embryonic mortality and the difference between ovulation rate and total number of embryos is considered to be early mortality.

Da Silva et al. (2018) related estimated breeding values (EBVs) for litter size (Topigs Norsvin, Vught, The Netherlands) to ovarian and embryonic characteristics of gilts at day 35 of pregnancy (see Table 1). In these gilts, an increase in one unit of EBV for litter size (i.e. one piglet) was related with a 1.12 increase in ovulation rate. There were no indications found that embryonic or placental characteristics were negatively related with EBV at this stage of pregnancy. Therefore, in these gilts, embryonic-placental units do not seem to be compromised at day 35 of pregnancy. It should be noted, however, that this group of gilts had a relatively high level of early embryo mortality, related with the used semen age, which may have negated potential negative effects of high ovulation rates on the embryonic-placental units.

Table 1. ß-estimates of the relations between estimated breeding values of gilts for total number of piglets born (EBV TNB), average piglet birth weight (EBV BW) and within litter piglet birth weight standard deviation (EBV BWSD) and ovarian and embryonic characteristics at 35 days of pregnancy (based on (Da Silva et al., 2018)

	EBV TNB, n	EBV BW, Kg	EBV BWSD, g
Ovulation rate, OR	1.12 ± 0.2	ns	ns
Corpus luteum weight, g	-0.11 ± 0.004	0.14 ± 0.04	0.0006 ± 0.0002
Total luteal mass, g	0.27 ± 0.12	ns	0.024 ± 0.01
Number of embryos	1.22 ± 0.4	ns	ns
Number of vital embryos	1.12 ± 0.3	ns	ns
Embryo weight	ns	ns	ns

ns - Non significant

In the same study, (Da Silva et al., 2018) analysed associations between EBVs for piglet birth weight and within litter variation in piglet birth weight with ovarian and embryonic characteristics at day 35 of pregnancy. The EBVs did not appear to be related with ovulation rate; so, selection for a higher piglet birth weight and lower within-litter variation in piglet birth weight did not affect ovulation rate. Interestingly, both the EBV for piglet birth weight and for within-litter variation in piglet birth weight had a strong positive association with another ovarian characteristic, namely average CL weight at day 35 of pregnancy (see Table 1).

In another study, Da Silva et al. (2017b) used transrectal ultrasound (TUS) to assess the average diameter of the 10 largest CL (5 per ovary) at day 20-30 of pregnancy, and related this to subsequent piglet birth weight and standard deviation in piglet birth weight (see Figure 4). Each extra mm in CL diameter was related with an increase in average piglet birth weight of 36 g and a standard deviation in piglet birth weight of 24 g. Moreover, an increase in EBV for litter size was related to lower CL weights (Da Silva et al., 2018)). Collectively, these data indicate that the selection for litter size resulted in smaller CL during pregnancy, which again is related to lower birth weights.

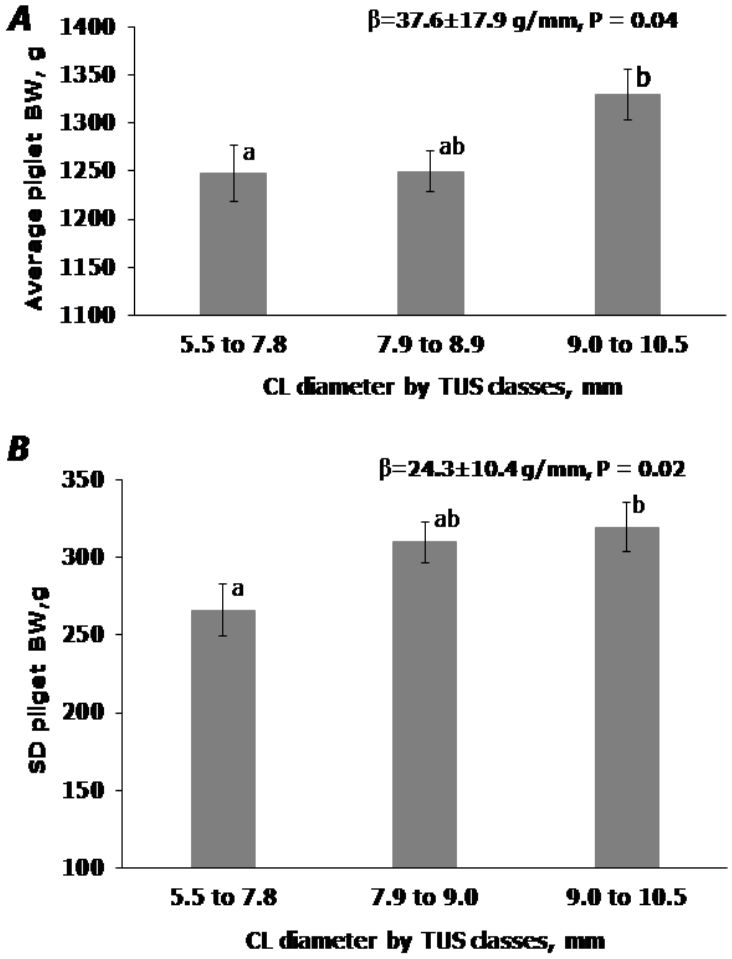

Figure 4. Effect of average CL diameter measured using transrectal ultrasound (TUS) at ~ day 30 of pregnancy [5.5 to 7.8 mm (n = 23); 7.9 to 8.9 mm (n = 47); and 9.0 to 10.5 mm (n = 30)] on BW of total piglets born [P = 0.04; corrected for litter size class P < 0.0001] (panel A) standard deviation (SD) of BW of the total piglets born (P = 0.02) (panel B). [ab] P<0.05. LSM±SE (from Da Silva et al., 2017b).

The next question that arises is: What could be the physiological mechanism explaining the relation between CL weight and litter characteristics? Average CL weight and variation in CL weight are positively correlated; so, a higher average CL weight is accompanied by more variation in the CL pool. As CL size has been related to the size of the pre-ovulatory follicles (e.g. Wientjes et al., 2012a), it is of interest to explore the possible contribution of these follicles on piglet birth weight (variation).

Pre-ovulatory follicle pool

The CL pool originates from follicles that are recruited from the antral follicle pool at weaning when the suppression of the hypothalamic-pituitary axis is released, and gonadotropin levels increase to simulate recruitment of small antral follicles to develop into dominant and ovulatory follicles. Considerable heterogeneity in size, morphology and endocrine status is seen in the pre-ovulatory follicles of gilts during oestrus (Hunter and Wiesak, 1990, Knox, 2005). The maturation of the oocyte within the follicle is highly dependent of the synthesis of steroid and (growth) factors by the surrounding granulosa and theca cells, as well as nutrients and hormones from the blood circulation. These all contribute to the composition of the follicular fluid. Extensive direct contact between the cumulus granulosa cells and the oocyte and exchange of metabolic intermediates further contribute to oocyte maturation and competence (from Costermans, 2020).

The heterogeneity in follicular (and oocyte) development and concomitant competence at ovulation has been related with variability in early embryonic development and subsequent mortality of the less developed embryos (Pope et al., 1990, Xie et al., 1990). Larger follicles (and thus larger CL (e.g. Wientjes et al., 2012a), may therefore represent better competent follicles at ovulation that release higher quality oocytes (Marchal et al., 2002) that develop into better

quality embryos. Indeed, at day 35 of pregnancy, a higher average CL weight was related with a higher vital embryo weight (r=0.17) and a higher vital implantation length (r=0.24) (Da Silva et al., 2018).

Thus, the association between CL size and piglet birth weight may find its origin in the association between follicle development and oocyte competence at ovulation. That this indeed might be the case is supported by studies in which insulin-stimulating diets during the period of antral follicle development, i.e. lactation and/or the weaning-to-oestrus interval (Van den Brand et al., 2006, Van den Brand et al., 2009) and weight losses during lactation (Wientjes et al., 2013) affected piglet birth weight and within-litter birth weight variation of the subsequent litter.

Recent studies in the PhD project of Natasja Costermans (Costermans, 2020) increased our understanding of early post-weaning follicle development. The aim of her thesis was to determine the parameters that determine follicular competence (i.e. the competence to release good quality oocytes) which may thereby contribute to piglet birth weight (variation). The first step in the thesis work was an attempt to relate the estimated breeding value for birth weight variation (EBV-LVR) of parity 3-5 sows to follicle development at weaning (Costermans et al., 2019c). Unfortunately, it was not possible to create a large contrast in EBV-LVR between the animals and no relations were found with follicle size or follicle variation. So, currently, direct information on relations between sow breeding values for birth weight variation and follicle pool characteristics is lacking.

To get a better understanding of the characteristics that determine follicular competence, follow-up studies investigated gene and protein expression of granulosa cells and follicular fluid composition in dependence of follicle size, follicle health (as determined by using a cleaved Caspase-3 staining of the granulosa cells, a marker for apoptosis) and the quality of the oocytes within the follicles (as determined by the morphology of the cumulus-oocyte-complexes (COCs)). Within the 10 largest healthy follicles on one ovary (the presumptive ovulatory follicle pool), it appeared that the granulosa cells of the smaller follicles expressed more cell proliferation markers and the larger follicles expressed more maturation markers (Costermans et al., 2019a) thus validating that differences in follicle size are related to follicle maturation, also at an early stage of development (at the day of weaning).

Subsequently, relations were studied between follicular fluid steroid profiles and oocyte quality of the presumptive ovulatory follicle pool. Sows with a high (> 70%) compared to low (< 70%) percentage of healthy COCs had higher levels of 17ß-oestradiol, 19-norandostenedione, progesterone and α-testosterone in the follicular fluid, while cortisol levels were lower (Costermans et al. 2019b). Furthermore, the granulosa cells of the sows with the higher percentage of healthy COCs had an increased expression of genes involved in steroidogenesis and follicular maturation. Interestingly, COC-health and follicle size were not related and sows with high vs low follicle size only differed in17ß-oestradiol content of the follicle fluid. Thus, at this early stage, not only follicle size, but also COC-health is an indicator of follicle competence.

Interestingly, the sows with a higher percentage of healthy COCs had lost less weight during lactation (7 ± 2 vs 12 ± 2 %) and had higher serum levels of IGF-1 at weaning (167 ± 15 vs 120±16 ng/ml). Surprisingly, differences in average follicle size between the sows were not affected by sow weight loss or IGF-1 levels at weaning, Thus, at this early stage, COC-health may be more affected by lactation energy balance than follicle size.

Relations between sow metabolic state and reproductive parameters are more commonly found in first-litter sows than in older parity sows as young sows that also need energy for growth usually have a lower feed intake capacity and therefore loose relatively more body weight during lactation, with consequent effects on follicle development and performance (e.g. Prunier et al., 2003). Wientjes et al., (2013) found that sows that lost more weight during lactation had higher birth weight variation in the next litter. Therefore, as a next step, follicle development was studied in weaned primiparous sows with different feed allowances during lactation (Costermans et al., 2019d). The sows received increasing amounts of feed up to 5.5 kg at day 9 of lactation and subsequently received either 6.5 kg or 3.25 kg up to day 24, the day of weaning. Follicle development was evaluated 48 h after weaning.

Feed restriction impaired follicle development in primiparous sows, as shown by the lower number of the larger follicles (Fig. 5a) and the reduced average size of the 15 largest follicles (Fig. 5b). It did not affect the percentage of healthy COCs at 2 days after weaning (Fig. 5c), but it did reduce follicle competence to support oocyte development as shown by the reduced COCs expansion after IVM and the reduced fertilisation rate and increased level of polyspermy after IVF of these oocytes (Fig. 5c). These consequences of feed restriction on follicular and oocyte development competence were associated to lower IGF1 levels in the follicular fluid and in serum of these sows.

The mechanisms behind effects of negative energy balance / weight loss on subsequent follicle competence are not entirely clear yet and may dependent on factors such as genotype or feed composition. Clowes et al. (2003) showed that selective protein losses in first litter sows (induced by moderate vs high protein levels in the lactation feed and a standard vs high body mass at parturition) reduced follicle size and follicle fluid oestradiol concentration at weaning. Costermans et al. (2019d) found that average follicle size was negatively associated to both loin muscle depth loss (ß = -0.85 mm/mm) and back fat loss (ß = -0.22 mm/mm) in the full-fed sows, but not in the feed restricted sows (see Figure 6; Costermans et al., 2019d).

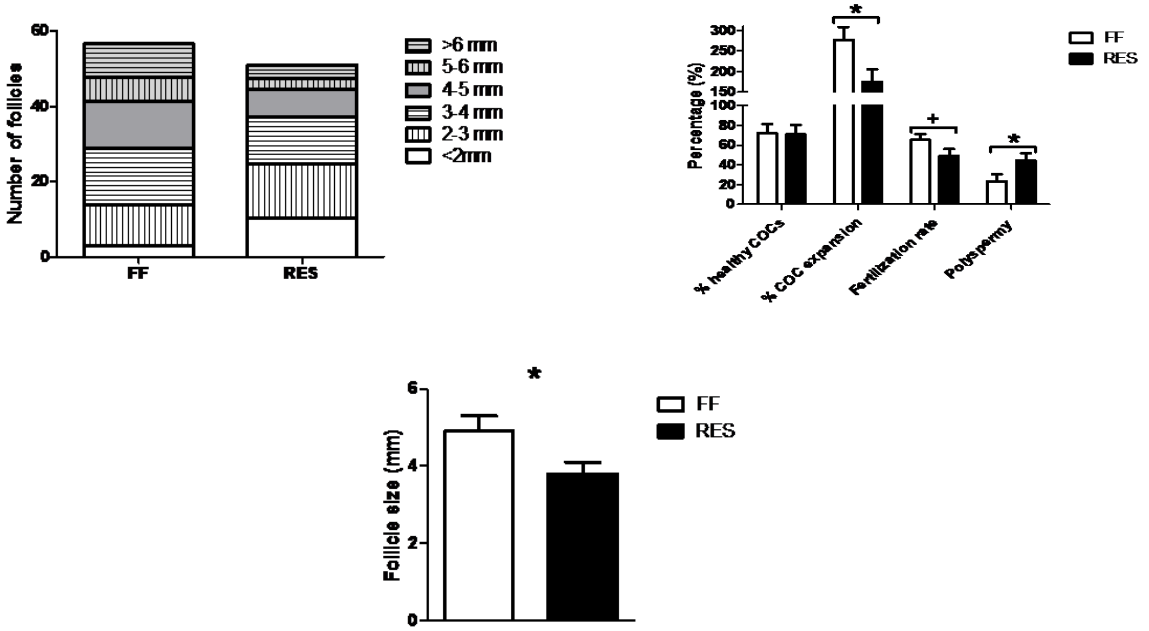

Figure 5. Follicle and oocyte development at 48h after weaning of primiparous sows that were full-fed or restricted-fed during first lactation A: total number of visible antral follicles in different size categories of both ovaries. B: Average follicle size of the 15 largest follicles of both the left and right ovary. C: Oocyte development at 48h and subsequent IVM and IVF results. (Costermans et al., 2019d).

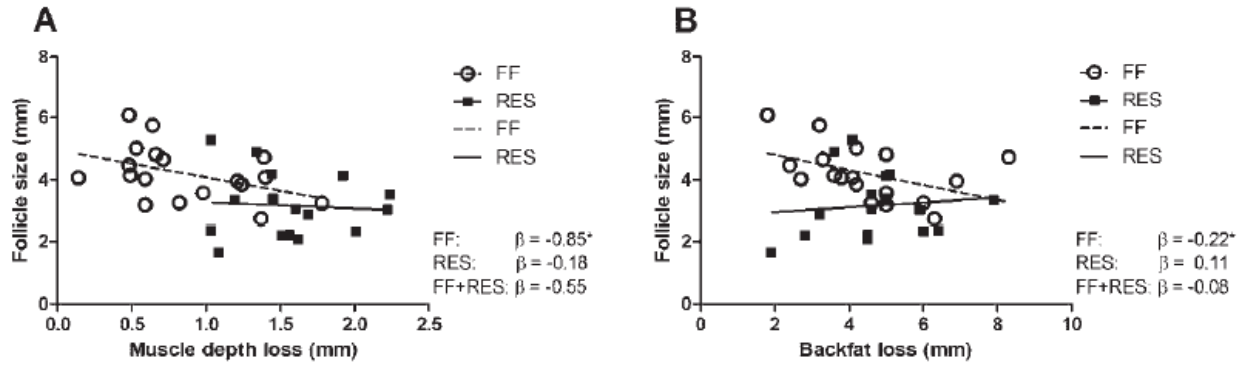

Figure 6. Relations between average follicle size (mm) of the 15 largest follicles of both left and right ovary 48h after weaning and A. Muscle depth loss (mm) during lactation and B. backfat depth loss (mm) during lactation. (Costermans et al., 2019d).

Thus, consequences of not only weight loss, but also specifically protein loss vs back fat loss need to be further evaluated, not only regarding follicle development and subsequent reproductive performance and litter quality, but also in relation to the suckling litter, as body condition losses are also related to milk production of the sow (Clowes et al., 2003; Costermans et al., 2020).

To summarize, higher litter sizes have come with lower piglet birth weights and more variable piglet birth weights, which subsequently are related to compromised post-natal survival and performance of piglets. Two hypotheses have been explored to explain the relation between litter size and (variation in) piglet birth weight; (1) Foetal-placental units are compromised due to uterine crowding, which is more predominant in sows with a high ovulation rate, and (2) selection for litter size has resulted in a more variable quality of the pool of ovulatory follicles. Mechanisms related to metabolic influences on follicle development require further investigation, especially in young sows.

Breeding and management solutions

In breeding programmes, gilts are not selected for only one trait (such as litter size) but for a number of traits that form the breeding goal. Based on the breeding goal, a selection index is created that uses multiple phenotypic traits that are important for the breeding goal (for example, birth weight and birth weight uniformity). Traits in the index receive a value based on their economic weight. Thus, potential negative effects of selection for litter size on (variation in) piglet birth weight or piglet survival are controlled by weighing these parameters (or correlated responses) in the selection index. Indeed, breeding companies have used piglet survival and/or (variation in) birth weight in their selection programs (reviewed by Zak et al. (2017).

Higher litter sizes have been achieved by increased ovulation rates and have resulted in higher levels of uterine crowding. It would be of interest to further investigate the level of uterine crowding (i.e. compromised foetal- placental units) in relation to ovulation rate at different stages of pregnancy. It may also be of interest to consider selection on traits that improve uterine capacity (Freking et al., 2016). Such strategies might not result in improved within-litter birth weight when its cause lies more in the variable pool of follicles at ovulation than in available uterine space. Also, information from Genome-Wide-Association-Studies (GWAS) (Calus, 2010) in such studies could elucidate the genetic background of found relations.

From a management point of view, possibilities to decrease variation in birth weight mostly seem to lie in optimizing nutrition and metabolic state in the early follicular phase. For example, Van den Brand et al., (2006) and Van den Brand et al., (2009) found that insulin-stimulating diets during lactation decreased birth weight variation and Wientjes et al., (2013) found that a higher weight loss during lactation and an increased weaning-to-pregnancy length was associated with subsequent birth weight variation. These effects seemed related with IGF-1 stimulating effects on follicle development. In the studies above, the reduction in birth weight variation seemed only moderate (approximately 2% reduction in coefficient of variation (CV)), but Wientjes et al. (2012b) found that every percent reduction in birthweight CV was related with a 1.08% reduction in early lactation piglet mortality, so also small reductions in variation can have substantial effects on survival. Similarly, each 100g increase in piglet birth weight was found to be related with a 3% decrease in piglet mortality rate.

As far as we know, no nutritional or management strategies during pregnancy have been effective in reducing birth weight variation.

Conclusion

Our knowledge on causes of (variation in) piglet birth weight has increased. We more and more realise that these changes to a certain extent are related to uterine crowding, induced by a higher ovulation rate and affected by the quality of both the ovulating oocytes and remaining corpora lutea. We also observe that there may not be many management options available to influence piglet birth weight (variation), although the follicle pool may be further optimised by creating the right nutritional – metabolic environment during and after lactation.

A point of attention is that relations between metabolic state and reproduction and birth weight are probably dependent on genotype and possibly other factors, which makes it difficult to provide general recommendations.

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Key production traits that determine sow lifetime productivity in commercial practice

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Introduction

Sow longevity is defined as the time elapsed from gilt entry into the breeding herd until removal as a result of culling or death. Increasing herd retention rate is important to maximize sow lifetime productivity. Higher removal rate implies an increase in the percentage of young females in the herd, a category with lower production potential, and a higher risk of early culling. Systematic gilt replacement is required for genetic gain. Still, it is essential to achieve a retention rate that allows the sows to reach their maximum production potential in the later parities. In this way, it is important to evaluate the reasons for removal and death to establish strategies for reducing involuntary culling and controlling mortality rates. Although reproductive failures persist as the main culling cause, its expressivity has been reduced. To increase sow retention rate, management intervention should start during gestation with a focus on improving the replacement gilt birth weight. Ensuring adequate colostrum intake is critical for achieving targeted pre- and post-weaning growth rate. Besides, approaches that improve gilt body development during the post-weaning phase and up to first breeding are critical steps to reducing culling for structural problems and low reproductive performance. In this sense, the use of gilt development units allows a constant flow of replacement gilts that meet the required parameters recommended by the different genetic suppliers. Because locomotor disorders are frequent reasons for early culling, inspection, and maintenance of the animal facilities should be prioritized in all phases. The opportunity to reduce sow mortality, culling for poor reproduction, and structural failure, resides in the ability to translate new research findings on gilt birth weight, growth, and body measures into improved lifetime pig production to the third parity.

Key production factors that determine lifetime productivity

Gilts are an important category in a swine breeding herd, representing around 15-20% of the breeding group females. Some key traits, such as gilt birth weight, age at puberty, lifetime average daily gain, and body weight at first mating, directly influence lifetime productivity.

1. Birth weight

The occurrence of low birth weight piglets is well documented in high prolific sows (Quesnel et al., 2008). The low weight at birth results in higher mortality rates until weaning and lower weights on the finishing phase. This phenomenon is also observed in gilts, leading to increased removal and mortality rates until selection at 170 d of age (Magnabosco et al., 2015). In this study, the authors observed higher mortality rates and losses until 170 days for female piglets born with less than 1000 g ($P < 0.0001$). Besides, when gilts were classified according to their birth weight into eight classes, the lightest birth weight class (410-990 g) was lighter at weaning than the heaviest birth weight class (1780-2400 g), and at selection (at 170 days of age), the gilts from the lightest class were lighter than the other classes.

Low birth weight also negatively influences female production and longevity. In the study of Magnabosco et al. (2016), the total number of piglets born ($P = 0.08$) and piglets born alive ($P = 0.028$) in the first farrowing were lower for gilts born with less than 1 kg. Moreover, these light gilts at birth produced fewer piglets ($P = 0.055$) over three parities than the heavier ones (Figure 1). The retention rate from selection up to third farrowing was not influenced by the birth weight. However, when the culling rate and mortalities from the pre-selection period were also considered, gilts with birth weight ≤ 1280 g remained in the herd for a shorter time than the heavier ones (Figure 2). Furthermore, cumulative mortality rate and cumulative losses until 170 days were higher for piglets born with less than 1000 g (Figure 3; $P < 0.0001$; Magnabosco et al., 2015).

Further investigations on litter-of-origin were studied by Vallet et al. (2016). The authors evaluated litter traits as sow parity order, birth weight, immunocrit, preweaning growth rate, and their relationship with body and reproductive traits at 260 d of age. All litter-of-origin traits were positively associated ($P < 0.05$) with female growth traits. The body weight at 240 d of age increased ($P < 0.01$) as the birth weight, immunocrit, and preweaning growth rate increased. However, sow parity order was negatively associated with the body weight; increased parity of birth was associated with decreased BW values ($P < 0.01$). In contrast to growth traits, reproductive traits were, in general, not affected by litter-of-origin traits. Age at puberty was associated with birth weight (positive; $P < 0.01$) and preweaning growth rate (negative; $P < 0.01$), indicating that age at puberty is delayed for heavier piglets at birth and slow growth piglets during the preweaning period. In this sense, more recently, Patterson and Foxcroft (2019) described a low litter

birth weight phenotype as a "litter" trait. This trait is repeatable over consecutive parities, and gilts born from a sow with a low birth weight phenotype have lower retention in the herd.

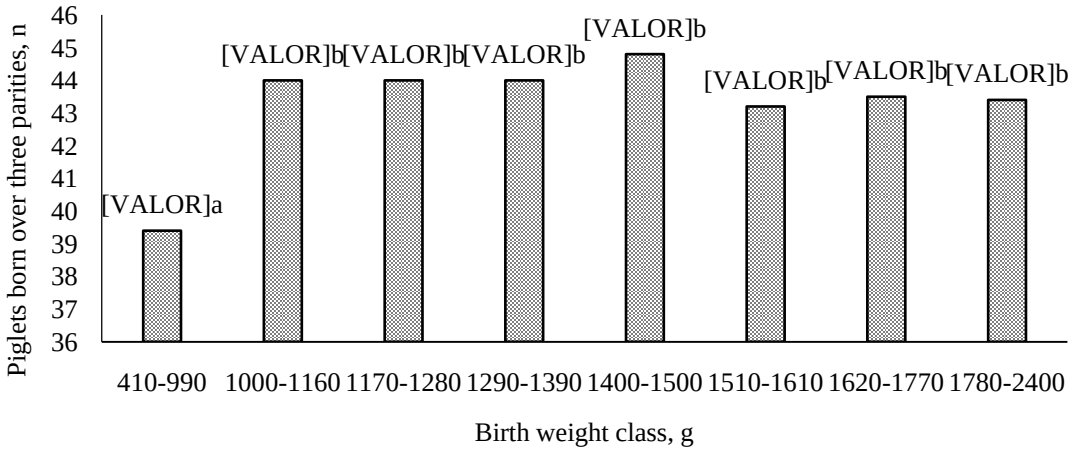

Figure 1. Total piglets born over three parities (n = 497) according to the birth weight of swine females. Bars with one letter in common are not significantly different (P > 0.05) - Magnabosco et al. (2016).

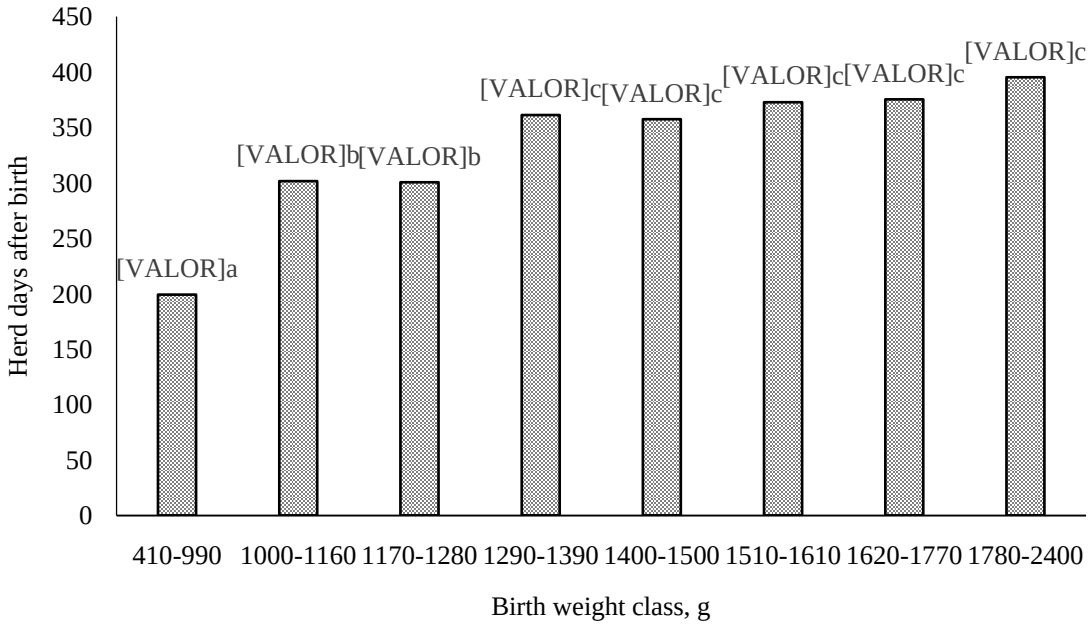

Figure 2. The number of herd days according to the birth weight of female swine. Days counted are from birth onwards, including all female piglets weighed at birth (n = 1495). Bars with one letter in common are not significantly different (P > 0.05) - Magnabosco et al. (2016).

In this context, reductions in puberty age might be accomplished by improvements in preweaning growth rates. Flowers (2009) observed that the number of suckling females within the litter in which gilts were raised (< 7 pigs or > 10 pigs) affected the sow longevity and reproductive performance. At the end of 6 parities, regardless of the age of puberty induction, significantly more sows raised in small litters (35%) were still in production compared with those raised in large litters (17%). The age in which puberty induction was initiated (140 or 170 days of age) also affected sow longevity and reproductive performance. Significantly more sows exposed to boars at 140 days of age (33%) remained in the herd compared with their counterparts given boar exposure at 170 days of age (16%). The effect of younger exposure to the boar and the size of litter in which gilt was raised were additive. In this way, 45% of the gilts exposed to boar with 140 days of age and raised on a litter with < 7 piglets were ready to be rebred after the 6th parity,

compared to only 10% of the gilts exposed to the boar at 170 day of age and raised on a litter with > 10 piglets. Even though the results of Flowers et al. (2009) are promising to early puberty stimulation (< 140 d), we should critically evaluate the logistic and the cost-benefit of anticipating this management.

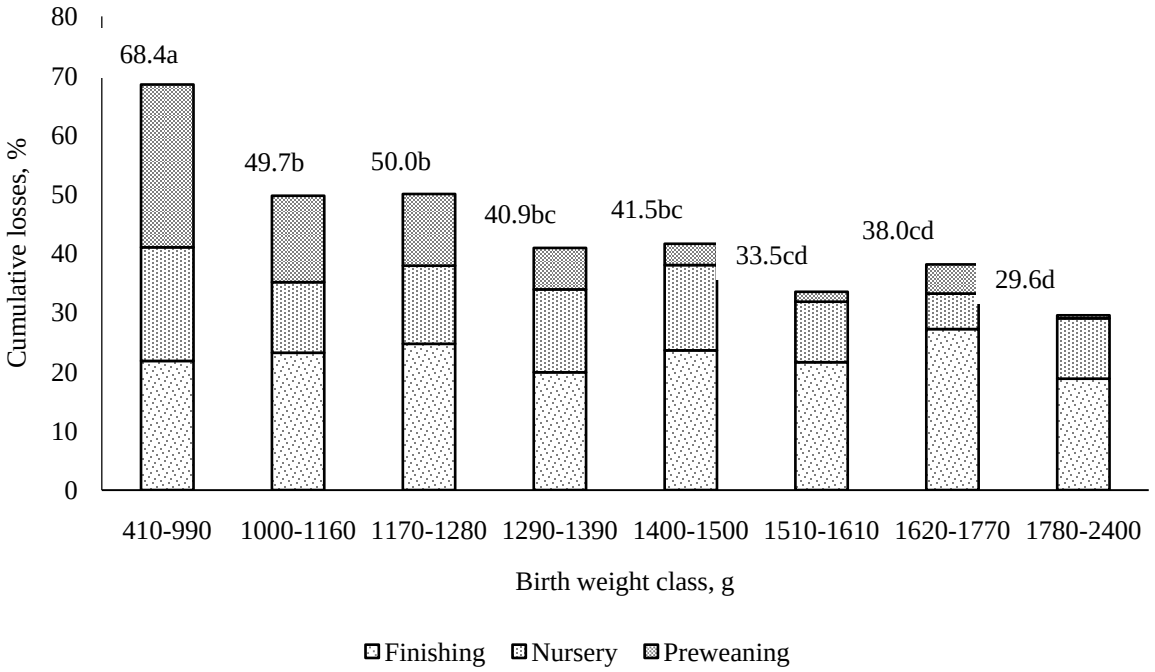

Figure 3. Culling and cumulative losses from birth until 170 days, according to birth weight classes of female piglets. Bars with one letter in common are not significantly different (P > 0.05) - Magnabosco et al. (2015).

2. Colostrum intake

In swine, colostrum can be defined as the secretion of the mammary gland in the first 24 h after farrowing being responsible for piglets' nutrition, thermoregulation, immunity, and growth. The amount and composition of colostrum produced may vary according to sow characteristics, such as endocrine, nutritional and immunity status, stress level, and heat stress (Quesnel & Farmer, 2019).

The average amount of colostrum needed by piglet is around 250 g. This amount reduces the risk of mortality (Figure 4), provides passive immunity and weight gain (Ferrari, 2013). Ferrari et al. (2014) demonstrated that colostrum intake is positively related to birth weight (P < 0.0001) and to serum IgG concentration (P < 0.0001). In this study, the mortality was affected by the interaction between birth weight and colostrum intake. For piglets with birth weight >1.3–1.7 kg, the probability of mortality was low regardless of their colostrum intake. The probability of mortality decreased as colostrum intake increased for piglets with birth weight between 1.1–1.2 kg and >1.2–1.3 kg, being necessary 200 and 250 g of colostrum, respectively, to reduce their probabilities to the same level observed for heavier piglets. Probabilities of mortality were similar among all birth weight categories when colostrum intake was >250 g (Figure 5).

However, at least one-third of sows do not produce enough colostrum to fulfill the requirements of their litter (Quesnel et al., 2012). The energy concentration of lactation diets is an important determinant of energy consumption and is typically modified by the use of fat, oils, or fibers in the diet (Tokach et al., 2019). The authors suggest that a lactation diet would be designed for optimal milk production and subsequent reproduction. Furthermore, a reduction in sow body weight loss and an improvement in litter growth rate during lactation would be expected. Thus, increased energy or amino acid intake in the few days before farrowing, during colostrogenesis, could be beneficial to the colostrum quality.

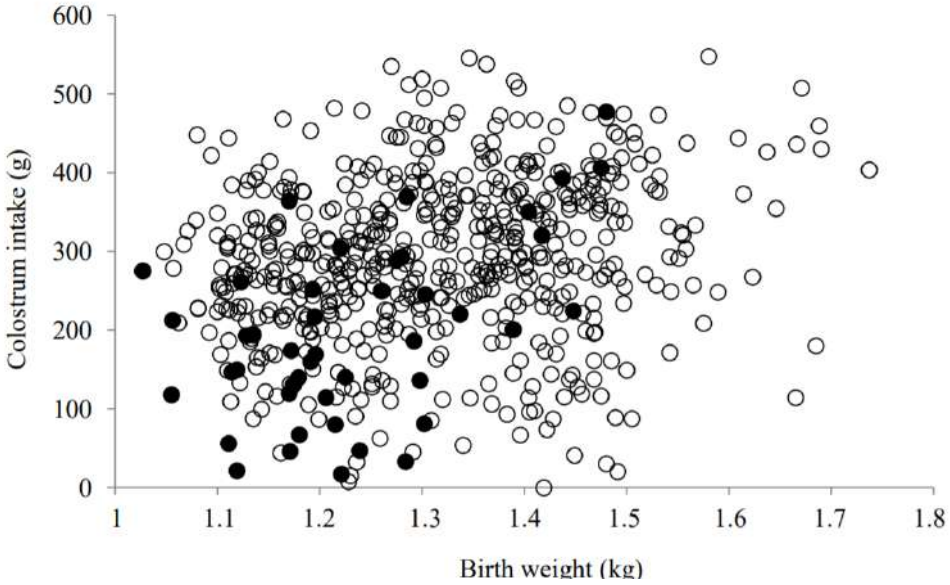

Figure 4. Piglets mortality until 42 days of age according to birth weight and colostrum intake. White circles represent piglets that were still alive at 42 days of age, and black circles represent piglets that died between 24 h after birth and 42 days of age – Ferrari (2013).

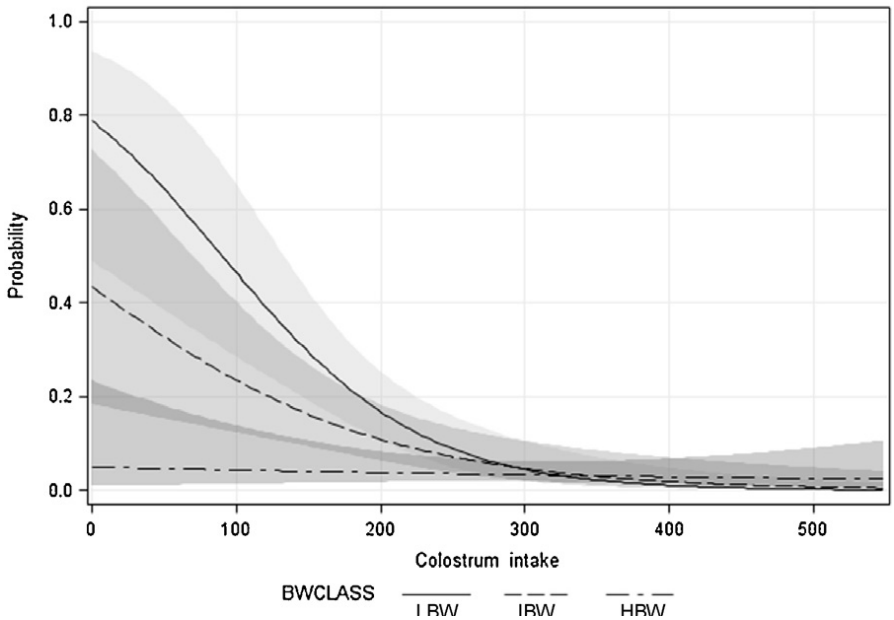

Figure 5. Probability of death until 42 days of age according to the colostrum intake and birth weight of piglets. LBW: piglets with a birth weight of 1.1-1.2 kg; IBW: piglets with birth weight >1.2-1.3 kg; HBW: piglets with birth weight >1.3-1.7 kg - Ferrari et al. (2014).

3. Lifetime Average Daily Gain

Lifetime average daily gain (ADG) influences the age of puberty, weight at selection, and weight at first artificial insemination (AI). These three factors together might compromise sow productivity and longevity. ADG affects total piglets born, piglets born alive, and weaning-to-estrus interval in the subsequent cycles. Increasing 100 g/d on ADG leads to an increase of 0.3 to 0.4 piglet in the litter and a decrease of 0.2 to 0.4 days on weaning-to-estrus interval (Tummaruk et al., 2001). Amaral Filha et al. (2010) categorized gilts in three classes (GI: 600-700 g/d, GII: 701-770 g/d, and GIII: 771-870 g/d) according to the ADG from birth to AI. The authors observed that GII and GIII gilts had higher total piglets born than GI gilts ($P < 0.05$), however, return to estrus and farrowing rate were not affected.

Kummer et al. (2009) compared two ADG groups of gilts (G1: 577 g/d, and G2: 724 g/d) and no differences on the number of ovulations, total and viable embryos at 32 days of gestation were observed (P > 0.05). During gestation, G1 gilts showed higher ADG and weight gain than G2 (P = 0.007). These data explain the positive correlation between gestation ADG and embryo survival (r = 0.29; P = 0.08) and show that lifetime ADG can be compensated during gestation, without affecting the reproductive performance. The study of Magnabosco et al. (2014) corroborates to Kummer et al. (2009); no difference was observed on farrowing rate and total piglets born on the first parturition between lifetime ADG classes (500-575 g/d; 580-625 g/d; 630-790 g/d). These results might be due to the achievement of target for weight at AI (130 kg at the first AI).

Recently, Walter (2018) evaluated gilts from selection to first AI after weaning, according to the lifetime ADG at selection with 140 days of age (G1 ≥ 480 - ≤ 530g/d; G2 > 530 - ≤ 580g/d; G3 > 580 - ≤ 630g/d; G4 > 630 - ≤ 810g/d). It was observed that the number of total piglets born, born alive, lactation length, weaned piglets, and weaning-to-estrus interval were not different among the groups. Besides, at insemination, it was observed that 94.67%, 92.67%, 91.13% e 91.04% of gilts from G1, G2, G3 e G4, respectively, showed ADG > 630 g/d. These data corroborate with Kummer et al. (2009), where gilts with low lifetime ADG until selection were able to compensate ADG from selection to AI.

Walter (2018) also investigated the retention rate and culling rate by reproductive reasons (Table 1). Gilts from G2 showed a lower retention rate from selection to 1st and 2nd farrowing when compared to gilts from G4. Regarding the culling rate by reproductive reasons, gilts from G2 showed a higher culling rate from selection to 1st and 2nd farrowing when compared to gilts from G3 and G4. Amaral Filha et al. (2008) observed that gilts weighing between 151-170 kg had a higher retention rate when compared to gilts weighing between 171-200 kg at the first AI, whereas no difference was observed for gilts weighing between 130-150 kg. The authors also observed that heavier gilts showed higher culling rates by locomotor problems, while no difference was observed on the culling rate by reproductive reasons among weight classes (Table 2).

Table 1. Retention rate and removals due to reproductive reasons until 3rd farrowing of gilts according to their ADG from birth to selection - Walter (2018).

Variables	ADG from birth to selection groups					
	G1 (n=800)	**G2** (n=978)	**G3** (n=916)	**G4** (n=607)	SEM	P-value
Retention rate, %						
Selection to 1st farrowing	92.0^{ab}	90.2^{a}	93.5^{bc}	95.1^{c}	1.08	0.0020
Selection to 2nd farrowing	79.1^{ab}	78.0^{a}	81.9^{bc}	83.9^{c}	1.53	0.0156
Selection to 3rd farrowing	70.9	69.3	72.8	72.3	1.71	0.3481
Culling by reproductive reasons, %						
Selection to 1st farrowing	4.6^{ab}	6.3^{a}	3.7^{b}	3.4^{b}	0.83	0.0221
Selection to 2nd farrowing	12.1^{ab}	13.5^{a}	9.8^{b}	10.0^{b}	1.27	0.0434
Selection to 3rd farrowing	14.8	16.7	13.8	12.9	1.47	0.1567

G1: ≥ 480 - ≤ 530g/d; G2: > 530 - ≤ 580g/d; G3: > 580 - ≤ 630g/d; G4: > 630 - ≤ 810g/d. SEM = standard error of the mean.Reproductive reasons included anestrus, return to estrus, abortion, and failure to farrow.

Table 2. Retention rate over three parities and culling reasons for gilts according to their weight at first insemination - Amaral Filha et al. (2008).

	Grouped according to weight at IA		
	130-150 kg (n=298)	**151-170 kg** (n=1007)	**171-200 kg** (n=421)
Retention rate over 3 parities, %	67.8^{ab}	68.5^{a}	61.1^{b}
Culling reason, %			
Reproductive	12.4^{a}	10.3^{a}	12.4^{a}
Locomotion	6.0^{a}	10.3^{b}	15.2^{c}
Death	5.7^{a}	4.4^{a}	5.2^{a}
Others	3.4^{a}	1.6^{a}	1.2^{a}

a, b, and c within the same row indicate significative difference (P < 0.05).

4. Nutritional management

Nutritional management is crucial for gilt development, ensuring satisfactory productive and reproductive performance. Each genetic company has a nutritional manual that contains the specifications to each phase of gilt development and gestation. In this scenario, flushing is a nutritional management applied before AI to increase the ovulation rate (Beltranena et al., 1991). The increased ovulation rate is important as it is the first step in establishing

litter size. Traditionally, nutritional flushing consists of an increase in feed amount or feed energy (Beltranena et al., 1991; Peruzzo, 2000) for at least 14 days before the first AI. Beltranena et al. (1991) showed that gilts fed additional 0.8 kg/day (Flushed) between the first and second estrus had an increase in the number of eggs ovulated compared to Control (14.0 vs. 12.0, respectively). Later, Peruzzo et al. (2000) compared the ovulation rate of gilts fed 2.0 kg/d vs. *ad libitum* and observed 1.6 more follicles ovulated in gilts fed *ad libitum*. These findings suggest that flush feeding gilts are crucial before insemination to increase the litter size.

In another way, the genetic selection to increase total piglets reduced the piglet birth weight and increased within-litter variability (Quesnel et al., 2008; Quiniou et al., 2002). During the final phase of gestation, piglets grow at an exponential rate (Ji et al., 2005). This phase was the target of several studies that evaluated the influence of different nutritional management in late gestation on piglet birth weight (Gonçalves et al., 2016; Mallmann et al., 2019). Mallmann et al. (2019) evaluated the effects of increasing the feed amount in late gestation of gilts (1.8, 2.3, 2.8, and 3.3 kg/d) on reproductive traits. Still, no differences were observed among treatments in the total number of piglets born and mummified fetuses. Tendencies for a quadratic effect of feed amount were observed for piglets born alive ($P = 0.079$), average piglet birth weight ($P = 0.083$), and litter weight ($P = 0.059$). Gilts fed on lower feed amounts during late gestation had reduced percentages of stillborn piglets in comparison to gilts fed with greater feed amounts. No differences in the subsequent cycle were observed among treatments for the farrowing rate, born alive, stillborn piglets, and mummified fetuses ($P > 0.05$). Furthermore, the retention rate over four parities and days until the female removal (Figure 6), number of total piglets born over four parities (Figure 7) were also not affected by the feed amount provided during late gestation in the first reproductive cycle.

Overall, the main goal during gestation is to achieve the requirements and avoid over-conditioned females. Females that are overfed during gestation will have their performance during lactation compromised. As observed by Mallmann et al. (2019), the heavier the gilts at farrowing, the lower was the colostrum yield and the voluntary feed intake. Moreover, the heavier the females at farrowing, the higher are the lactation body losses. Lactational catabolism is associated with impairs on subsequent performance. So, during lactation, the main goal is maximizing the feed intake to sustain milk production without excessive body mobilization (Menegat et al., 2018). The recommendation for the lactation period is providing *ad libitum* feed. It is also important to mention that primiparous sows will need special care since these are not able to ingest the amount of the necessary nutrients to achieve the requirements. This approach is also valuable for the period of heat stress, where the females reduce the voluntary feed intake by 3.7% for each 1 ˚C that the temperature increases above 25 ˚C (NRC, 2012; Menegat et al., 2018).

During the wean-to-estrus interval, the objective of the feeding program is basically to improve the ovulation rate and, if necessary, to recover the body condition in females that had higher body losses during lactation. So, providing higher feed amounts during this period may help the recovering, which in turn needs to be extended during the early gestation period (Menegat et al., 2018). Whereas, for females in a good body condition, providing higher feed amounts is not necessary. Recent studies showed that there are no benefits to reproductive performance by providing higher feed amounts or more energetic diets y (Graham et al., 2015; Gianluppi et al., 2019). Gianluppi et al. (2019) compared females (primiparous and multiparous) fed 2.7 or 4.3 kg/d, with lactation or gestation diet, and no improvements on the farrowing rate and litter size were observed.

Replacement gilt flow is a key step for herd health, productivity, and longevity. Thus, the farm needs to be prepared to receive and introduce this category within the reproductive herd. Even though this is primary management in the farms, it is not always well managed by the farm team either for the lack of training, lack of information, or management failures. In this context, there are some recommendations that the farm manager can implement to have better results, healthy animals, and, consequently, a more productive herd.

The logistic of the herd to receive the replacement gilt is extremely important. Once the farm established the breeding group, it is necessary to guarantee the infrastructure to receive and prepare the gilts for breeding. In this context, the main step is having a schedule for gilt management. The farm manager needs to know the correct time and age to receive the gilts at the farm, and when to start the puberty stimulation to introduce them to the reproductive herd. In this way, the arrival at the farm and gilt preparation for breeding are crucial to obtain healthy and productive females. It means that the replacement gilts might be able to be bred with the scheduled breeding group. In consequence, the retention of old or unhealthy females will not be necessary to achieve the insemination target.

In our current situation in Brazil, gilts arrive at the farms with 140 to 160 days of age to be bred at 200 to 210 days of age, based on a target weight. However, this recommendation may vary among genetic companies. Gilts will stay in the gilt development unit (GDU) for about ten weeks, in pens or in individual crates. For each breeding group, new gilts will be introduced to the GDU. Thus, the GDU needs to be dimensioned, considering the number of animals housed, to support the replacement animals to the farms. One of the main management of gilts arrival is their acclimation. Each farm needs to have its acclimation protocol, based on the health status of each herd.

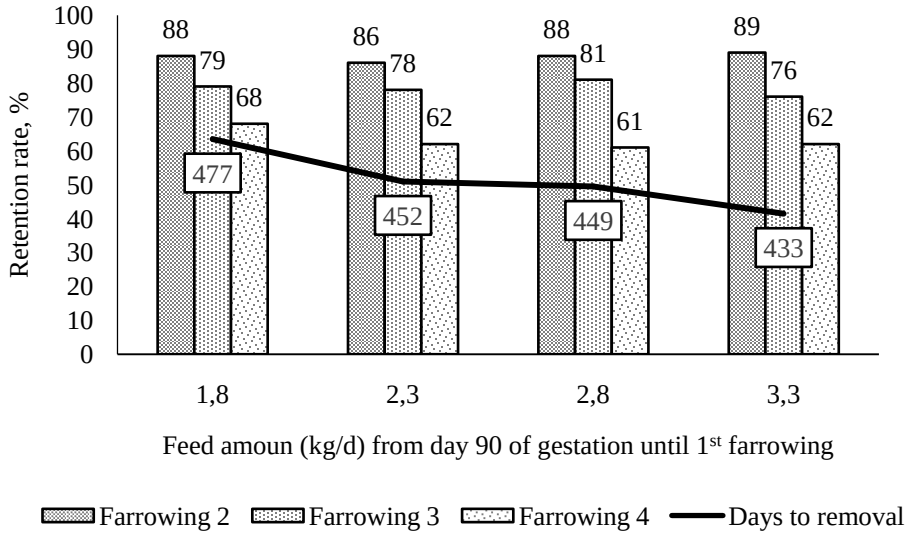

Figure 6. Retention rate and days to removal over four parities of gilts fed with different feed amounts during late gestation of the first reproductive cycle - Mallmann et al. (2020) – data not published.

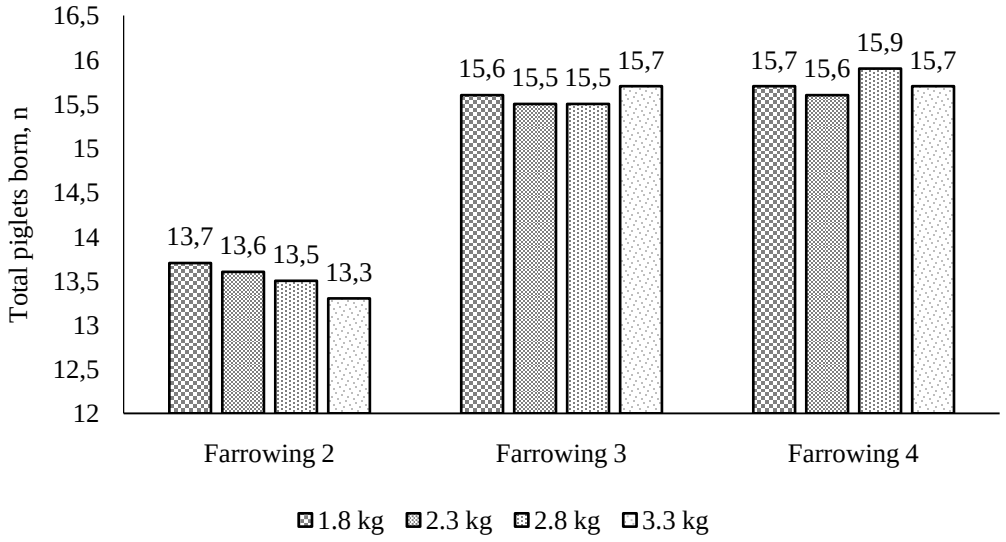

Figure 7. Total piglets born over four parities of females submitted to different feed amounts during late gestation in the first reproductive cycle - Mallmann et al. (2020) – data not published. Adequate replacement gilt flow in the herd.

After receiving the gilts, under our current sanitary situation in Brazil (PRRS-free), they need to be prepared in a few weeks to be bred. This management includes feeding program according to the genetics recommendations (aiming target for weight and body condition score at first AI); individual identification sheet per gilt and pen; selection of gilts with good locomotor and general health status; satisfactory puberty induction management; following an adequate vaccinal protocol; individual adaptation at stalls (if necessary); flush feed before breeding for two weeks; attention with estrus detection for breeding and attention on the quality of semen doses. These managements are essential to have gilts in good corporal, reproductive, welfare, and health status. By following these key steps, we can expect good reproductive results at the first and subsequent farrowing.

Increase retention rate and controlling mortality in the breeding herd.

As mentioned before, in a healthy herd, there is always a necessity of a good flow of replacement gilts. However, it is necessary to control the mortality rate to not lose sows with still a good reproductive and genetic potential. In Brazilian herds, the average mortality rate is around 8%; however, in the best 10% Brazilian herds, the mortality rate is about 4.3% (Bierhals et al., 2019). These data suggest that there are a lot of opportunities to decrease the sow mortality rate, and this can be accomplished mainly with good management strategies. This management

includes: facilities climatization; floor quality (minimizing locomotor problems); adequate flow of replacement gilts (avoiding inadequate sow retention); avoid overweight sow herd; daily inspection of animals (identify overfed and thin animals; check every animal every day: locomotor, health, appetite); daily inspection on water quality; trainned caretaker (responsible for medication and management of hospital pens) and a precise recording on mortality causes.

Conclusion

Sow and gilt management are the keys to the reproductive success of the herd. Sow longevity and reproductive performance are related to female early lifetime development (Figure 8). In this way, management protocols that ensure the selection at birth of female heavier than 1 kg and a good colostrum management (the intake of at least 250 g of colostrum), ensuring an adequate preweaning development, are crucial on gilt development. Consequently, selecting gilts with good corporal development, lifetime ADG $\geq$ 630 g/d at the selection, and between 130-150 kg at first AI, improves subsequent retention in the herd. However, an adequate growth curve until puberty and a target weight at first insemination are not enough. It is important to monitor the first parity sow until the first weaning. Thus, the female early reproductive lifetime would be well carried, and the retention chances increase as well as the productivity. A correct replacement gilt flow guarantees parity order structure within the herd, intending to maintain the genetic potential. The opportunity to reduce the mortality rate just using management protocol is an easy approach for the farm to have great results and ensure sow longevity within the herd.

Gilt management – schematic view

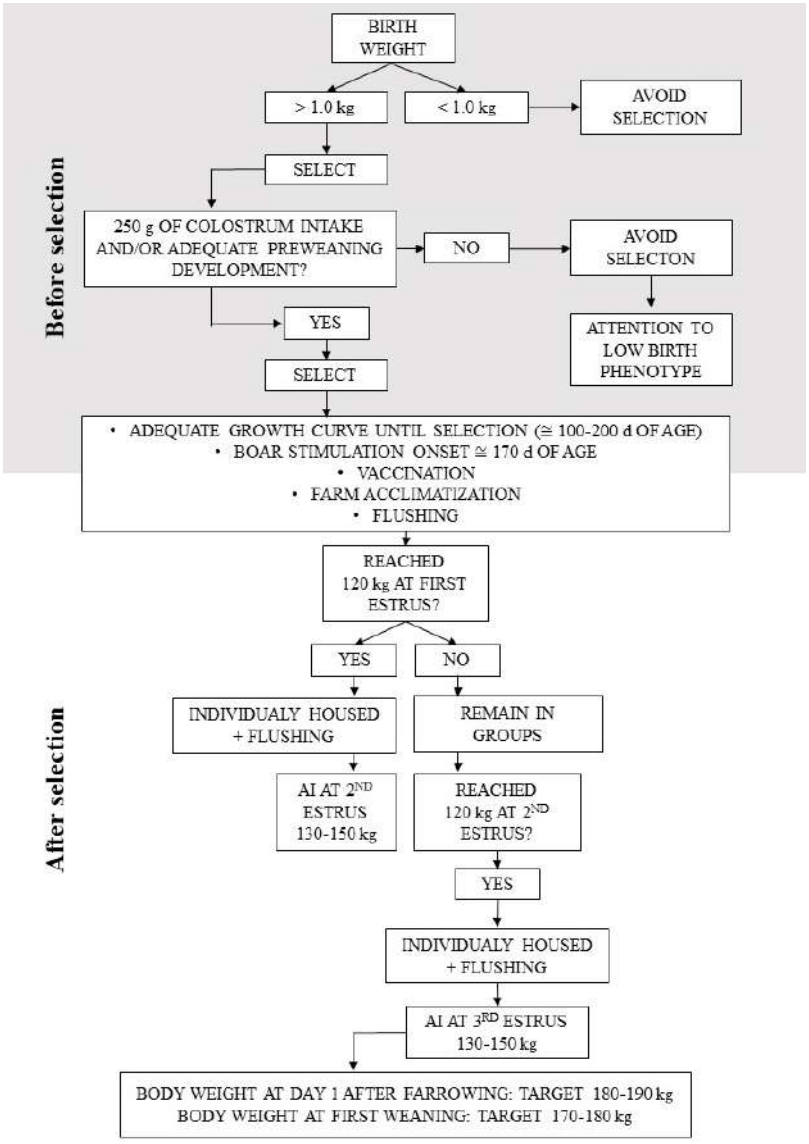

Figure 8. Schematic view of gilt management from birth to first weaning. The recommendations may vary among

genetics lines.

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Antimicrobial resistance, a one welfare challenge for pig farming

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One of the most pressing problems that has attracted considerable publicity in the last few years is the prospect of widespread multidrug resistance leading to a breakdown in human healthcare systems throughout the world. The O'Neil report (O'Neil et al 2016), estimates "that by 2050, 10 million lives a year and a cumulative 100 trillion USD of economic output are at risk due to the rise of drug- resistant infections". The O'Neil report recommends a reduction in the unnecessary use of antimicrobials in agriculture and their dissemination into the environment.

There is no doubt that the use of antimicrobials is a driver for antimicrobial resistance (AMR). When a mixed population of susceptible and resistant bacteria are exposed to antimicrobials the resistant organisms will survive and the susceptible ones die – this is simple natural selection. Consequently, the use of antimicrobials in agriculture will lead to increased AMR. One of the key questions is the degree to which agricultural use of antimicrobials contributes to the human burden of AMR.

The recent WHO guidelines on use of medically important antimicrobials in food-producing animals (Anon, 2017) and the systematic reviews (Tang et al, 2017) that informed these guidelines highlighted the dearth of good quality evidence on the impact of agriculture on human AMR. Only 13 studies were found to be of sufficient quality to be used to make meaningful conclusions and almost all these studies were performed in Western Europe and North America. There is a pressing need to address this knowledge gap, particularly in a wider context that should include low and middle income countries where high antibiotic use and limited regulation place them in the front-line in the global fight against drug-resistant infections. Research is currently underway in my own and other labs across the world to address knowledge gaps highlighted in the OIE Strategy on Antimicrobial Resistance and the and the Prudent Use of Antimicrobials (Anon, 2016).

There are two good examples which highlight the potential threat of agricultural sources of resistant bacteria to human health. These are livestock-associated methicillin resistant *S. aureus* (LA-MRSA) and colistin resistance in enterobacteriaciae.

Staphylococcus aureus is an opportunistic pathogen that normally colonizes the host asymptomatically, but can cause a variety of pathogenic infections. Some *S. aureus* clones are more successful human pathogens than others, and some show a high degree of host specificity for different animal species (Sung et al, 2008). Recently, a specific lineage belonging to multi-locus sequence type (ST)398, most likely of human origin, has spread among livestock globally, acquired methicillin resistance and is now transferring back to humans leading to both colonization and disease (Price et al, 2012). Pigs constitute a large reservoir for LA-MRSA ST398 and contribute to an ongoing spread and genetic adaptation in Europe and North America. Although the ST398 lineage of *S. aureus* was the first designated to be 'livestock-associated' in Europe and the US, broader investigations have confirmed that MRSA of other MLSTs (e.g., ST9, ST5) also occur in swine populations (Sun et al, 2015). Furthermore, the relative prevalence of these lineages, and subtypes within lineages, appears to vary geographically (Battisti, 2010; Sun et al, 2015). While ST398 variants have been predominant in studies of pigs in Europe, ST9 has been identified as the predominant LA-MRSA lineage in most Asian countries (Chuang, 2015). Both ST9 and ST398 LA-MRSA have been reported in both China and the UK suggesting that both lineages may be established in both countries (Hadjirin et al, 2015; Yan et al, 2014). Of considerable concern is the report of reduced vancomycin susceptibility in porcine ST9 MRSA isolates from pigs in China (Kwok, 2013). The identification of LA-MRSA ST9 isolates (in China; Yu et al 2014) and ST398 isolates (in the Europe; Ward et al 2014) from human infections and carriage clearly indicating the threat to human health of these lineages.

The molecular epidemiology of LA-MRSA is required in order to confirm transmission pathways and identify significant virulence factors. Comparative genomic studies of LA-MRSA have identified some phage associated genes that appear to be correlated with virulence in humans, but no genes of importance for successful colonization or infection in livestock or other animals have been identified (Uhlemann et al, 2012). A greater understanding of the pathogenicity and transmission of LA-MRSA requires further investigations into the survival mechanisms utilized by these lineages. This understanding will inform the development of strategies to reduce the impact of LA-MRSA on the colonization of livestock and human health. However, there is no doubt that LA-MRSA from pig farms leads to human carriage and disease.

Carbapenem-resistant bacteria are a big problem in human medicine. Colistin (polymyxin E) is a polycationic peptide antimicrobial that was isolated and characterized in 1949. For many years it was largely abandoned in human medicine due to toxicity issues, but widely used in veterinary medicine. In recent years it has become a "last-line" therapeutic drug, for the treatment of infections in hospitalized patients caused by carbapenem-resistant Gram-negative

bacteria (Nation et al, 2017). The discovery of plasmid-mediated mobilized colistin resistance (mcr) genes originating from pigs in China has become a major issue (Liu et al, 2016). At the time of writing there have been relatively few reports of multi-drug resistant bacteria with colistin resistance that are causing widespread problems, but the gene is now widely distributed and the potential for such infections is growing. It is highly likely that the use of colistin as a growth promotant contributed to the selection pressure that led to the emergence of the *mcr* gene.

In both of these examples there appears to be little effect on animal health. *S. aureus* is not a primary pig pathogen and has little opportunity to be a secondary pathogen compared to human medicine. It appears that livestock act as a reservoir of MRSA that add to human MRSA carriage rates, which then may lead to disease as carriage is the biggest risk factor for MRSA infection. Similarly, there have been no reports of treatment failures due to colistin resistance in pig farms but bacteria harbouring the *mcr* gene do escape from farms into the environment and into the human population.

Colistin resistance is an example of a low probability event (considered as likelihood at a single point of time) with the potential of a high cost to public health. LA-MRSA is an example of a high probability event with a relatively low cost to public health (again looking at single point events). There are concerns that either of these scenarios could be repeated with the acquisition of new AMR resistance genes in bacteria which are pathogenic to people originating on farms.

It is important to appreciate that it is likely that in comparison to the contribution that the misuse of antimicrobials by the medical profession when treating people has made to AMR, the contribution from agriculture probably represents a very small proportion. A mathematical modelling study from van Bunnik and Woolhouse (2017) concludes that “Our results suggest that, for a wide range of scenarios, curtailing the volume of antibiotics consumed by food animals has, as a stand- alone measure, little impact on the level of resistance in humans.” This may reflect the current situation, but this does not excuse the historic overuse or misuse of antibiotics in some agricultural sectors, on the other hand it does provide some context.

Excessive and injudicious use of antimicrobials in farming is unsustainable. It has long been argued that overmedication of livestock has been used to compensate for poor welfare and bad husbandry. The overarching aim of good antibiotic stewardship is to use as little as possible, but as much as necessary. Preventive medicine approaches include the use of vaccines and making husbandry changes that reduce incidence of disease. Clearly, reducing incidence of disease leads to a reduction in the need to medicate with antimicrobials. While the pig industry has been historically good in areas such as biosecurity it has striven to increase productivity levels with little regard to other effects. Freedom from disease is one of the tenets of good animal welfare, and agricultural sustainability is essential to support rural economies and feed our growing population. A coordinated and proportionate response to the AMR crisis from the industry and government is required to this one-welfare issue.

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Towards the development of epigenetic marks of stress and welfare in farm animals

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Abstract

Epigenetic mechanisms have been extensively studied in many organisms in relation to diverse biological questions. In production animals, however, despite the enormous implications that epigenetics could have for welfare and production aspects, its application is incipient. One of the concerns regarding production practices is the stress that animals experience while in captivity. Despite regulations around the globe, stressful practices are very common in some regions. In animals undergoing stress, a plethora of hormonal responses are produced. Because of this, short-term stress in animals is usually determined by cortisol and epinephrine levels. However, stress hormones can show an acute but not a sustained exposure to stressful conditions. An important challenge is to determine the history of the exposure of an organism to stress in live specimens. We postulate that epigenetic marks in peripheral cells (e.g., blood cells, buccal swabs) can serve as epigenetic biomarkers of a history of stress with enormous potential to be converted in an application in real situations within the production environment, particularly in hens and pigs. Epigenetic marks of stress would greatly improve the ability of authorities, welfare inspectors and veterinarians to objectively diagnose welfare in farming systems.

Introduction

While meat production generates food and a livelihood for billions of people around the planet, it is also associated with environmental and health consequences (OECD 2016). Among production animals, chickens and pigs are species of enormous economic importance for humans, with chickens being the most consumed meet source in the world (13.8 kg/capita in 2016) followed by pigs (12.3 kg/per capita in 2016) (OECD 2016). Given the cultural and economic importance of these meet sources and the growing concern about climate change and animal welfare, it is extremely important to create tools that foment sustainable production practices in the meat industry. One of the concerns regarding production practices is the stress that animals experience while in captivity. Although different levels of regulation around the globe exist, some detrimental practices are common in some regions, such as beak trimming to avoid pecking, exposure to random illumination patterns to promote accretion, or exposure to arousing odors, loud noises, too low or too high temperatures, among others (MORGAN AND TROMBORG 2006).

In animals undergoing stress, hormonal responses are produced such as changes in testosterone, norepinephrine, epinephrine, prolactin, adrenocorticotropic hormone, and cortisol (HENRY 1992). Because of this, short-term stress in animals is usually determined by cortisol and epinephrine levels (ISHIBASHI *et al.* 2013; MULLER *et al.* 2013). However, the release of stress hormones can show an acute but not a sustained exposure to stressful conditions (HENRY 1992). Thus, an important challenge is to determine the history of the exposure of an organism to stress in live specimens. Epigenetic marks in peripheral cells (e.g., blood cells, buccal swabs) could serve as epigenetic biomarkers of a history of stress (PROVENCAL *et al.* 2012; WANG *et al.* 2012) with enormous potential to be converted in an application in real situations within the production environment.

Epigenetic mechanisms involve chemical modifications of the DNA that can regulate gene expression and be maintained after cell divisions (SKINNER *et al.* 2010). Epigenetic mechanisms are fundamental players in the development of phenotypes, and are sensitive to environmental influences (JIRTLE AND SKINNER 2007). Thus, external influences that affect early life stages (pre- and post-birth) can have dramatic consequences on epigenetic processes that ultimately shape the adult phenotype (GUERRERO-BOSAGNA AND SKINNER 2012). Several factors are among the environmental influences reported to interfere with epigenetic mechanisms during early development, among them endocrine disrupting (SUSIARJO *et al.* 2013) or inorganic (KILE *et al.* 2014) chemicals, nutritional compounds (DOLINOY *et al.* 2007; GUERRERO-BOSAGNA *et al.* 2008) or stressful conditions (FAGIOLINI *et al.* 2009).

Although research on epigenetics has permeated many fields of biological research, from molecular biology to evolution (STEIN AND DAVIS 2012), and has employed a variety of organism models (e.g., laboratory rodents (DOLINOY *et al.* 2007; GUERRERO-BOSAGNA *et al.* 2008; SUSIARJO *et al.* 2013), invertebrates (LYKO *et al.* 2010), plants (MANNING *et al.* 2006) or yeast (ZHANG *et al.* 2013)), the understanding of epigenetic mechanisms in farm animals is minimal. The consideration of epigenetics is essential to fully understand molecular mechanisms related to the phenotypes of production animals, and thus can lead to uncover the molecular basis of behavioral traits not well predicted by genotype, such as those related with detrimental behaviors in farm animals (e.g., tail biting in pigs, feather pecking in chickens).

Epigenetics can also provide mechanistic cues in processes of *transgenerational epigenetic inheritance* (TEI), which involves effects of environmental stimuli on exposed individuals and on their unexposed descendants (GUERRERO-BOSAGNA AND SKINNER 2012). TEI is thought to be mediated by environmentally-altered epigenetic marks (epigenome) in the gametes that are transmitted across generations. Transgenerational effects have been reported in a variety of organisms, including lab rodents (ANWAY *et al.* 2005; GUERRERO-BOSAGNA *et al.* 2010; GUERRERO-BOSAGNA *et al.* 2012), fish (BAKER *et al.* 2014a; BAKER *et al.* 2014b; BHANDARI *et al.* 2015), quails(LEROUX *et al.* 2017), ducks (BRUN *et al.* 2015) and chickens (GOERLICH *et al.* 2012). Although initial evidence of TEI was generated by environmental exposures acting in early developmental stages, recent reports in rodents have shown that juvenile or adult exposures could also affect the germ line epigenome with consequences in future generations (CARONE *et al.* 2010; RODGERS *et al.* 2013; DIAS AND RESSLER 2014).

In chickens, early social isolation has been shown to affect the HPA axis and gene expression in the thalamus/hypothalamus of the offspring (GOERLICH *et al.* 2012). Transcriptomic changes in the hypothalamus of chickens raised with unpredictable light patterns are also observed in their offspring (NATT *et al.* 2009). These effects observed in parents and their offspring suggest transmission of stress-induced germ line epigenetic alterations to future generations.

Overview of ongoing research

We previously showed that epigenetic marks in red blood cells (RBCs)of chickens reflect their previous rearing condition, in cage or open aviaries (PERTILLE *et al.* 2017). These conditions associate with different stress levels and cognitive abilities. Two questions originated from this experiment that were studied in the present investigation: i) are epigenetic marks in RBCs associated with specific stressors? and ii) how long in life these epigenetic alterations persist? In follow up experiments (unpublished data) we exposed 4-day old male chickens to social isolation stress incrementally for three weeks. This stress has been previously shown to have long-term and transgenerational effects. We then collected RBCs immediately after the stress treatment ended and six months after, in a completely renewed RBC population. We performed DNA methylation analysis in a reduced fraction of the genome and interrogated whether correlations exist between the DNA methylation changes observed in these two time points. Our analyses revealed that even in the control group old individuals tend have hypermethylated sites in relation to young individuals. Interestingly, DNA methylation is incrementally disrupted in response to the stress as animals age, predominantly through hypermethylation, while the enriched environment produces predominantly hypomethylated sites in old individuals in comparison to young ones.

This abovementioned experiment was performed in parallel at Linköping University in Sweden (SW population) and the Brazilian Agricultural Research Corporation (Embrapa Swine and Poultry National Research Center; BR population). The aim was to identify stress associated with DNA methylation profiles in RBC across these populations, in spite of the variable conditions to which birds are exposed in each facility, and of chickens coming from independent lineages. Interestingly, we found three significant ($P < 0.05$) differentially methylated regions overlapping between the BR and SW lineages. This is of high relevance because these are putative epigenetic biomarkers of stress in production animals from different lineages, breeding programs and biomes.

These results in chickens prompted us to expand our research towards pigs. In collaboration with the group of Dr. Adroaldo Zanella at the University of Sao Paulo, we are investigating the relationship between stereotypies observed during pregnancy in saws and the brain methylome of the offspring. In collaboration with Dr. Linda Keeling from the Swedish Agricultural University we are investigating how the genome and the brain methylome varies among performers, victims and non-involved individuals in relation to tail biting

Our current studies are giving epigenetics a central role as a tool to evaluate detrimental exposures in the production environment. We expect the results obtained in the near future will represent a tipping point in relation to the use of epigenetic tools within the production context. The results emerging from our ongoing experiments should pave the way for the development of toolboxes based on epigenetic marks that will result in improved animal welfare. The aim is that epigenetic toolboxes will identify if animals have been exposed to detrimental conditions (e.g., stress, exposure to chemicals) in the production environment. This will allow for rapid evaluation of previous exposures or health/welfare status using peripheral cells from live animals. The potential incorporation of these tools in the production environment would help to transition animal production practices towards the new demands in terms of animal welfare and sustainability.

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Understanding the behavioural needs of growing and finishing pigs and how we can meet those needs when designing future housing systems

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Animal emotions – and what about the pig?

With some notable exceptions Darwin's 1872 book on expressions of emotions in man and humans, 'feelings' or emotions have historically been viewed as non-scientific and not a subject fitting for scientific enquiry. However, during the last decades we have seen a resurrection of scientific interest in the field of mechanisms of emotion, not at least because of the increasing prevalence of emotional control deficits in human mental disorders. There are many definitions of emotions, most of them originating from human psychology. For example, Cabanac (2002) defines emotion as "any mental experience with high intensity and high hedonic content characterized as pleasure or displeasure". He proposed that pleasure can be used to equate the strength of motivational drive that is linked to each regulatory need (Cabanac, 1992). Any behaving organism, being human or animal, must rank its priorities, and thus pleasure (or lack of suffering) can be a currency to achieve this ranking. Nevertheless, some authors still claim that consciousness is a prerequisite to experience emotions (e.g. Damasio et al., 2000), and there is still an ongoing debate concerning animals` capacity to be aware of their emotions (e.g. Salzen, 1998). Several authors within the field of evolutionary biology have hypothesised that emotions are processes evolved to help animals to avoid harmful stimuli and seek valuable resources or reward (e.g. Panksepp, 1994; Cardinal et al., 2002; reviewed by Paul et al., 2005). This view has been summarized by Balcombe (2009) in his paper on animal pleasure with: '*evolutionary principles predict that animals, like humans, are motivated to seek rewards and not merely to avoid pain and suffering*'. Today, in the human literature it is well described that emotions are essential for biosocial perceptions, serving to cast a selective attentional spotlight on some experiences (prioritizing versus distractions, incoherence versus focus, selective etc), that they are a motivator of behaviour. Positive emotions will enable an individual in decision making, i.e. predicting risk taking evaluations (Levine, 2007). In principle, pleasure can be categorized into four types (Duncker, 1940; reviewed by Cabanac, 2002).

1. Sensory enjoyment or displeasure i.e. to enjoy the stimulus or the consequences of behaviour;
2. Aesthetic enjoyment i.e. to strive for a better understanding;
3. Desire (e.g. for a steak, a book, a love, etc.), not a reaction but the fulfilment of a need;
4. Pleasure in achievement/problem solving, dynamic joy of succeeding, in victory.

At the simplest level, usually approach/avoidance can be used to measure valence (pleasant- or unpleasantness). Nevertheless, more complex behaviours such as play, and exploration are more adequate measures of positive affect because these behaviours usually only occur in the absence of fear and anxiety and after basic needs are fulfilled. Play behaviour of young animals as in humans stimulates motor and social skill development, which are basic components to secure a future positive, stable behavioural mental development and stimulates advanced coping abilities (reviewed by Estevez et al., 2007). The pleasure component of a play behaviour helps to motivate the animal/human to continue this behaviour like in explorative behaviour, solving a challenging task, enjoying something tasty or comfortable (i.e. tactile stimuli), or enjoying success of victory. For pigs, provision of straw, peat or other sources of rooting material may stimulate this behaviour as well as exploration, and for a longer period than simple play objects such as chains or tyres. Rooting behaviour is a highly prioritized behaviour in pigs. Although chopped straw is commonly used to stimulate this behaviour, peat, mushroom compost, sand, sawdust, wood shavings, branches, beets and silage are all ranked above straw in preference tests and operant conditioning tests (e.g. reviewed by Studnitz et al., 2007). The less manipulative objects like beams, ropes, tyres or chains are least preferred. This preference or ranking of different rooting materials may also be manifested through the different level of pleasure or indicators of positive affect that these elements create. Thus, studying behavioural indicators when presented different rooting materials, may serve as an efficient way of evaluating different sources of rooting materials as well as other sources of environmental enrichment. Since emotions are short-lived responses, spontaneous behavioural expressions in animals such as ear or tail movements, body postures or even facial- and eye expressions during play and exploration, are highly relevant to be carefully studied. Furthermore, the variety of vocalisations (in terms of intensity and category; e.g. Manteuffel et al., 2004) can be relevant indicators of emotions. Can behavioural indicators of affective states be viewed as animal signals? Well, what is the point of showing joy, satisfaction or happiness if you cannot share it with someone? And what is the point of showing distress or misery if you were all alone and did not think that there was someone around that could take care of you or help you out of this difficult situation?

"Fulfilment of a need" and the "success in solving a learning task" form the basis for initiating a positive emotional state (i.e. positive mental balance). Cognitive challenges presented in connection with foraging behaviours initiate positive emotional states in the animals and thus improve welfare (Puppe et al., 2007). An increase in environmental complexity that creates novelty and challenges in terms of increased movement, problem solving, or social stimuli also have positive effects on brain function and cognitive skills in general (Radak et al., 2001; Würbel, 2001). According to Manteuffel et al. (2009), 'instrumental behaviour includes motivation for a specific reward, anticipation of its successful acquisition and positive appraisal'. Siegford et al. (2008) demonstrated that when pigs were successful in solving cognitive challenges during early life they showed reduced fear responses as adults, suggesting that positive experiences reduces negative emotions. Spatial learning trials in a maze have been successfully used in many species including and pigs (e.g. reviewed by Murphy and Arkins, 2007; Siegford et al., 2008) and pigs appear to solve these challenges rather well.

Emotions activating the limbic system has an impact on modulated immune response (Haas and Shauenstein, 1997). As greater negative affect in humans is linked to heart disease, cancer, arthritis and diabetes, greater positive affect is linked to lower hospital re-admission, reduced risk of stroke and a lower mortality (review: Consedine and Moskowitz, 2007). Humor and laughter also have a positive impact on recovery of cancer patients (Mahony et al., 2002). Although, health effects are documented in humans, their underlying biological mechanism has been scarcely studied in animals (Ernst et al., 2006). In principle, the effects of positive and negative psychological experience may depend on the degree of success or frustration.

The importance of environmental enrichment

Environmental enrichment can be defined as species-relevant modifications of animal environments resulting in improved biological functioning (modified from Newberry, 1995). Farm animals have strong behavioural needs that are important to satisfy in order to achieve a positive mental balance and thereby more healthy animals. Pigs are no exception from the rule. The need to play and explore by rooting in social groups of young pigs, are both highly prioritized activities and extremely important for their behavioural development into robust individuals. For instance, serum content of brain derived neurotrophic factor, BDNF, that plays an important role in neural survival, growth and plasticity, increases when young pigs are provided with foraging substrate block as enrichment during lactation or after weaning compared to pigs housed in barren environments (Rault et al., 2018). Greater concentration of BDNF in the brain is associated with an improved cognitive function and a greater stress resilience and could thus be an important welfare indicator.

Provision of straw, peat or other sources of rooting material may stimulate play behaviour as well as exploration, and for a longer period than simple play objects such as chains or tyres. In our recent project (Ocepek et al., under review), we offered weaned pig litters 10 l of rooting material (silage, long-stemmed straw, peat, or a combination of all three), twice daily, and compared this with groups that were not offered any rooting material. Behaviours considered indicative of positive affective states in this context (exploration, play, tail curled, tail wagging), as well as behaviours associated with harm (ear/tail manipulation, aggression, tail down), were quantified from video recordings. The peat and combo conditions resulted in higher levels of exploration, play, tail curled and tail wagging, and lower levels of ear/tail manipulation, aggression and tail down, compared to control, with the silage and straw conditions mainly giving intermediate results. Pigs showed more exploration, tail curled and wagging after than before provision of silage, straw, peat and combo, whereas an increase in play after material provision occurred only in the peat and combo conditions. Similarly, Marcet-Rui et al. (2019) found that straw was efficient in reducing harmful behaviours, but did not affect indicators of positive affective states, such as tail movement. In our study (Ocepek et al., under review), exploration occurred at similar levels on Day 1 and 4 of exposure to the peat and combo conditions whereas it declined across days in the other conditions. Comparatively, ear/tail manipulation and aggression increased in the silage condition. Peat as a single material and even more a combination of three rooting materials, substantially reduced negative behaviours and increased the occurrence of positive behaviours indicative of a more positive affective state in these pigs. The combo treatment also led the pigs to contact a novel person and a novel object faster, and enhanced the ability to collaborate with a pen mate in a small task to access rooting material from a closed box with a lid (Woldsnes et al., 2019). Therefore, we can expect stronger, positive and more long term effects on welfare if we provide pigs with a combination of materials at least twice a day. The amount of material should be sufficient, and for instance smaller amounts of 100 grams or less with chopped straw, tested experimentally, do not give clear welfare effects (Amdi et al., 2015). In Norway, it is manifested in the regulations that all pens need a small amount of sawdust to keep pens clean, but this is not considered as rooting material. The less manipulative objects like beams, ropes, tyres or chains are least preferred. Wooden sticks and especially spruce are more attractive than chains and other objects, but still not successful in reducing harmful behaviours such as ear chewing or tail biting (Chou et al., 2018). The preference or ranking of different rooting materials may also be manifested through the different level of pleasure or indicators of positive affect that these elements create. Thus, studying behavioural indicators when presenting different rooting

materials, may serve as an efficient way of evaluating different sources of rooting materials as well as other sources of environmental enrichment.

Although only marginal physical (i.e. addition of toys, extra feeder) and social (i.e. larger groups) enrichment can make a temporary difference for pig welfare in terms of less body lesions caused by aggression, more exploratory behaviour, and an increased ease of handling (Tönepöhl et al., 2012), the goal for future housing systems should be to create more complex, heterogenous environments, including access to an outdoor areas (Figure 1), that can stimulate the pigs to a much larger extent than what we see in indoor systems today. It is not necessarily a discussion about indoor vs. outdoor systems since there are also many welfare challenges with an outdoor system, and theoretically it is possible to make a fully enriched environment indoors. However, the cost of housing pigs in larger groups with substantially more space per pig, with more equipment (although larger groups require fewer pen divisions) and material to stimulate activity, will cost more indoors than outdoors. We therefore predict that future housing for pigs will include access to controlled, outdoor areas that are protected from wild boars and pathogens.

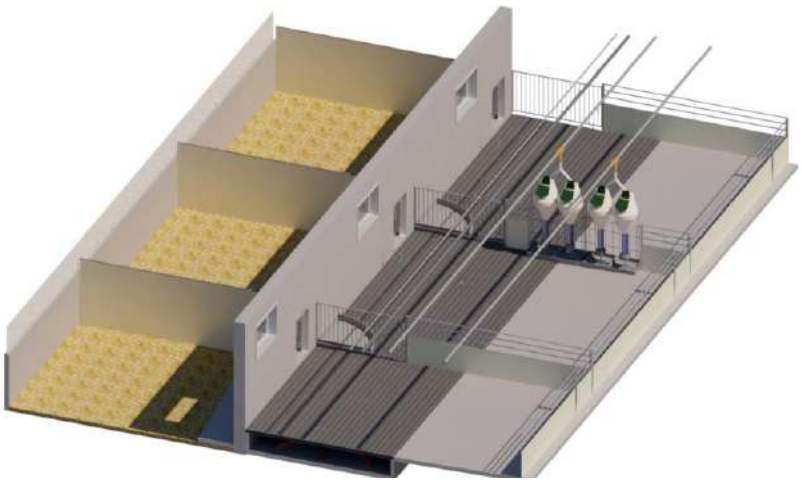

Figure 1. Future housing of fattening pigs with access to a controlled outdoor arena with deep litter/straw bedding. The illustration is made by May Helen Gryte, Fjøssystemer A/S, Norway.

In an indoor environment, the pens are often barren and with too little stimulation between the time of feeding/drinking and resting. It appears that nothing can compete with access to straw bedding or other types of deep bedding areas as the most enriched environment for pigs (e.g. Scott et al., 2006), and should preferably be included in future housing systems to a larger extent if access to outdoors is not an option. For pigs to be able to fully express their behavioural repertoire**,** and especially play and explore, enough space is needed. Thus, we expect positive effects of environmental enrichment to become more evident when enough space is available. More specifically, growing pigs with more space available (1.0 m^2/pig) explores rooting material (i.e. chopped straw or maize silage) more and manipulate pen fittings less than pigs with little space (0.64 m^2/pig; Jensen et al., 2010). There is also a mounting body of evidence that suggests regular exercise improves brain function and causes structural, biochemical, and physiological adaptations via different pathways (Radak et al., 2007).

One of the most convincing studies regarding the effects of enrichment on production performance and meat quality was done by Beattie et al. (2000). In their study, pigs were reared from birth to slaughter in either barren or enriched environments. The barren environments were defined as intensive housing (slatted floors and minimum recommended space allowances) and the enriched environments contained extra space, and an area with peat and straw provided from a rack (i.e. not only one source of enrichment). Not surprisingly, this more complex, enriched environment reduced time spent inactive and time spent involved in harmful social and aggressive behaviour while increasing the time spent in exploratory behaviour. During the finishing period, mean daily food intakes were higher and food conversion ratios were lower for pigs in enriched environments. Growth rates were also higher for pigs in enriched environments during this period which resulted in a higher carcass weight. Pigs from enriched environments also had greater levels of backfat than those from barren environments. Finally, meat from pigs reared in barren environments was less tender and had greater cooking losses than pork from pigs reared in enriched environments. Whether pigs are housed in partially slatted or straw bedded systems does not seem to affect meat quality variables in other studies that much even though the deep bedding systems usually increase activity and locomotion (reviewed by van de Weerd & Day, 2009), but many social factors in interaction with type and amount of environmental enrichment could potentially affect meat quality variables. This must be studied in a more detailed and systematic way including the entire housing design making more heterogenous environments where the pigs can be occupied with different positive

activities, but then again, we should not expect that all improvements in welfare are translated into improved production performance.

Social dynamics and the effects of animal density and group size

Pigs prefer to be kept in their stable litter groups as long as possible and mixing within a closed space is almost with no exception associated with more aggression and body lesions caused by fighting both in young pigs (e.g. Andersen et al., 2004) and in adult sows (e.g. Andersen et al., 1999). Therefore, the more stable we can keep the groups and the less mixing we must do, irrespective of animal density or group size, the better it is for pig welfare. Behavioural plasticity allows animals to change strategies and adapt more easily to varying environmental (social and physical) conditions within a confined group (Estevez et al., 2007). We developed a theoretical model that describes how aggression among unacquainted, weaned pigs is a function of group size when keeping space per animal constant (Andersen et al., 2004). We proposed that as the number of potential competitors increases, more individuals will not benefit from getting involved in costly fights as the probability of monopolizing resources will decline with increasing group size. Small group sizes of 6-12 individuals per pen have significantly more fights per pig than groups of 24 pigs, and in the largest group size a higher proportion of individuals did not engage in aggressive conflicts at all (Andersen et al., 2004). Other studies show similar results (Nielsen et al., 1995; Turner et al., 2001) or that the aggression remains relatively constant with increasing group size (Schmolke et al., 2004). Overall, it appears that pigs not only are less aggressive in larger groups, but also shift to a low-aggressive social strategy when they are moved from a small (18 pigs) to a large group (108 pigs), and that pigs raised in smaller groups tend to be more aggressive when meeting unacquainted pigs in a new group situation (Samarakone & Gonyou, 2009). In the literature we find terms such as 'futures contracts for nonaggression' (Pagel and Dawkins, 1997) and the 'tolerant system' (Estevez et al., 1997) to describe this phenomenon of reduced aggression in larger groups. Pigs kept in groups of 20 compared to 5, 10, or 15, also made fewer but longer visits to the feeder and ate more per visit and faster than pigs kept in the smaller groups (Nielsen et al., 1995), clearly showing that their feeding strategy was also changed with group size. Results from free-range conditions suggests that group size also may depend on the activity of the pigs: pigs tend to split into smaller subgroups during the day when they are foraging, while they are united as larger groups during rest at night (Rodrìguez-Estèvez et al., 2010). This may be a good anti-predator strategy for the pigs under free-range conditions but could also very well reflect similar behavioural needs under indoor conditions. However, there is little evidence for more sub-grouping in larger groups of pigs kept indoors but rather that they tend to be more dispersed than in smaller groups (Turner et al., 2003). It is thus likely that sub-grouping relates to resource distribution rather than the group size per se. One possible, negative side effect of larger groups with more total space for instance on deep straw bedding could be that weight gain might be reduced compared to smaller group pens or that it demands a higher feed consumption to keep a similar weight gain (Turner et al., 1999). A reason for higher feed consumtion can be an increased activity in larger groups with more total space, as it has been documented that pigs from large groups (80 pigs) in pens with deep straw bedding are standing more, show more locomotion and interact more with their environment (i.e. social interactions and exploration) than pigs in conventional, smaller pens with a group size of 15 (Morrison et al., 2006). However, nor the latter study or older studies (Nielsen et al., 1995), find differences in growth performance between group sizes, suggesting that other factors in the environment may interact with group size and pen system. Meat quality data shows that loins from the deep-litter pens with large groups also had a lower pH, more purge loss, more glucose in purge and were lighter in subjective color. On the other hand, there was no difference in tenderness, juiciness or overall desirability detected by a trained sensory panel. We do not yet know what the optimal group size for pigs is regarding optimizing social behavior, welfare, growth performance and meat quality, but there are many welfare benefits of larger group pens as it is more stimulating for the pigs. Effects of group size needs to be studied systematically in combination with for instance pen design, animal density, feeding system, environmental enrichment, and resource distribution (i.e. number and location of feeders and drinkers, rooting material, as well as attractive resting places) as these factors most likely will interact. Our current field survey and project on Norwegian fattening pig welfare and productivity will cover all these factors.

Regarding animal density, finishing pigs (around 75 kg) housed at 0.8 m^2 show more negative social behavior, has more lesions on all parts of the body including ears, are less clean and has a higher body surface temperature than those housed at space allowances of 1.2 or 1.6 m^2 per pig, respectively (Fu et al., 2015). Interestingly, the number of positive social behaviours in this study was greatest in the intermediate density. Comparatively, Norwegian legislations state a minimum space requirement of 0.65 m^2 for pigs in this weight category (www.Mattilsynet.no) and could be considered too small to ensure good welfare conditions. Which is the most important factor for pig welfare, space or enrichment? Beattie et al. (1996) attempted to answer this question many years ago by comparing the following space allowances for newly weaned groups of 6-week-old pigs: 0.5, 1.1, 1.7 or 2.3 m^2 per pig, all being enriched with free access to the substrates peat and straw, and added a fifth group with the largest space allowance but with no enrichment. The results showed that there was less exploration of substrates and more inactivity in in the enriched group with the smallest space allowance and the non-enriched group with the largest space allowance. Pigs moved more when given

greater floor space in enriched pens, but duration of harmful, social behaviour was greater in the barren treatment with the largest space allowance than in the four enriched treatments with different space allowances, suggesting that enrichment is more important than space allowance per se but that a minimum amount of space is needed for the pigs to be able to explore and interact in a positive way. From this study it was concluded that enrichment played a greater role in determining pig behaviour than floor space allowance, which makes sense since the pigs motivation to move around and engaging in positive activities are goal directed. For instance, pigs explore to find food and chewable objects, and space to move around in itself is not enough to satisfy that need. However, to be able to offer more optimal environments for the entire growing and finishing period, we need to combine both physical and social enrichment (i.e. larger groups, group composition, stable littermate groups) with a wider range of space allowances and pen designs as resource distribution, location of rest areas etc. are also likely to affect this picture to a large extent. If pigs could work to get access to attractive resources such as a self-administered rooting material dispenser, pigs would be even more stimulated than when giving them free access. Overall, there is not enough scientific documentation of the effects of space allowance in combination with factors such as source and frequency of enrichment and pen design.

Digital technology and deep learning methods for automatic recognition of pig behaviours – a powerful tool for animal welfare assessment

New digital technology to recognize positive and negative behavioural indicators will become an important welfare assessment tool in the future. In our recent project "DigiPig," our goal is to produce a digital surveillance system for behavioural recognition of pigs based on video sequences and machine learning, combined with an "app" where the farmer can keep track of a welfare protocol as well as management routines to assess welfare status at the farm over time, and to have immediate feedback on his or hers everyday routines. The concept will include both welfare and productivity measures, and the goal is to increase and simplify the everyday awareness of the condition of animals in the herd, and to give the farmer an efficient tool for this purpose. Making online animal welfare courses is a good thing, but in the end, it is the farmers everyday practical routines and handling of the animals that is going to make a difference. Therefore, we must develop a system that is motivating and user friendly for the farmer, at a low cost, work efficient, and leading to a welfare friendly, sustainable animal production. Based on annotating 600 images from 2D video recordings of groups of weaned pigs provided with rooting substrate, we have developed a program that recognizes each individual pig with a precision of 96%, the tail with 77% and heads with 66%, respectively (Figure 2). Surprisingly, the tail was easier to identify than the head. This is also a positive result for the future development of the system, as we predict that a straight tail is associated with a negative affective state, a curled tail with a neutral to positive state and a wagging tail to an excited (aroused), positive state. This was also demonstrated in our recent study on weaned pigs (Ocepek et al., under review). The tail is thus considered one of the major indicators of the welfare conditions of young pigs. However, as we would like to have a screening of the situation both on individual and group level, we consider the following behaviours to be important:

- Pig tail: straight hanging down, curled, wagging
- Individual deviation from behavioural synchrony (as pigs in a group usually have a synchronous activity pattern (i.e. eating, resting etc.)
- Solitary and social play
- Exploration
- Fighting
- Ear and tail biting
- Pig vocalisations: Low-frequent grunts vs. pig screaming

Most of these behaviours are associated with a certain body posture that can be recognized in the similar way as the sow behaviours in the recent studies mentioned below. Deviation from synchrony and vocalisations can be done on group/pen level whereas the others are on individual level.

Several sensor modalities are now available for automatic monitoring of behaviour, for instance deviations in drinking and feeding, frequency of coughs and vocalisations have been registered by using such systems combined with automated alerts sent to mobile phones (Matthews et al., 2016). Deviations from behavioural synchrony in groups of pigs could also potentially be interesting as they tend to show very synchronous activity patterns and the individuals deviating from this pattern could potentially have some problems. Similarly, sound recording on group level could reveal if pigs are screaming to a large extent or if the group sound is dominated by low-frequent grunts associated with a positive mental state. Programs using facial recognition of individual pigs show an accuracy of 96.7% from 1553 images (Hansen et al., 2018). However, if one wants to monitor several pens with many pigs in each and get an insight into their welfare status, we need cameras from above with a slight side angle, and thus it would be easier to recognize pigs based on shapes rather that faces as their face are usually oriented towards the ground. The face will thus be less visible than their body posture or shape, tail or ears.

Figure 2. The annotations were created with Labelbox which is a customizable collaborative online tool. Labelbox is free for academic research. The image material was extracted from 400 hours of video.

Deep learning and machine vision approach for posture detection of individual pigs has great potential as a welfare assessment tool. Two-dimensional imaging system along with deep learning approaches can successfully be utilized to detect the standing and lying (belly and side) postures of pigs under commercial farm conditions (Nashiramadi et al., 2019). Data from different commercial farms were used for training and validation of the proposed models. Experimental results show that for instance the R-FCN ResNet101 method was able to detect lying and standing postures with a mean precision of more than 93%. This is extremely interesting as both positive behaviours, such as play and exploration, and negative behaviours such as in aggressive conflicts are associated with certain postures that most likely can be recognized from images. Similarly, Yang et al. (2018 a), used deep learning for automatic recognition of sows' nursing behaviours in 2D images, with an accuracy of 97.6%, sensitivity of 90.9% and specificity of 99.2%. Faster R-CNN and ZFnet has been applied to recognize individual feeding behaviours of pigs (Yang et al., 2018 b), where each pig in the barn was labelled with a letter. Their proposed method was able to recognise pigs' feeding behaviours with an accuracy of 99.6%.

Image analysis techniques using fully convolutional networks (FCNs), appears to be one of the most promising methods of automatic recognition of sow behaviours from video sequences. In a study of lactating sows (Yang et al., 2020), temporal features that evaluated the temporal motions of the animals were extracted, and these spatial and temporal features were then put into a hierarchical classifier for behavioural recognition. Based on 468,000 frames of 3 sows, accuracies of behavioural classification compared to manual scoring was: 97.49% for drinking, 95.36% for feeding, and 88.09% for nursing, respectively. We will look into the details of this method for the further development of DigiPig.

Eliminative pig habits and pen design – how can we achieve a dry and a clean pen?

Understanding eliminative (urination and defecation) pig habits is important to achieve a dry and clean pen. Inappropriate eliminative behaviour causes fouling of pen resting (lying) area, and this has a negative effects on the environment, for instance regarding ammonia emission (Ocepek and Škorjanc, 2016), the cleanliness of pigs and pens (Andersen and Pedersen, 2011; Bøe et al. 2019), pig and human health (Urbain et al., 1994).

Pigs are some of the cleanest animals as they distinguish between areas for resting and eliminating (Whatson, 1978; Stolba and Wood-Gush, 1989). For resting areas, pigs prefer to choose an area with a solid floor (Aarnink et al., 1997), in close proximity to feeders (Baxter, 1982), without disturbance from other pigs (e.g. the neighbouring pens; Hacker et al., 1994), and with a warm surface (Marx and Buchholz, 1989). To keep the resting area clean, pigs choose to eliminate as far away as possible from it (Stolba and Wood-Gush, 1989; Ocepek and Škorjanc, 2016), and in a separate area when this is available (inner vs. outer; Guo et al., 2015; Ocepek and Škorjanc, 2016; Ocepek et al., 2018). They prefer to eliminate on slatted floor (Aarnink et al., 1997), in cold (Banhazi, 2013), bright (Taylor et al., 2006), wet (Baxter, 1982) areas, in close proximity to walls or in the corner of their pens (Baxter, 1982; Bate et al., 1988). Pigs have also been observed to eliminate close to drinkers (Ocepek et al., 2018). The placement of drinkers in the outside area compared to the inside area resulted in more than a 30% decrease in the eliminating on resting area and a 20% increase in eliminating on slatted floor, thereby reducing fouling, less time needed for manual cleaning, and improved pig welfare. This information is of great interest if we want to plan future housing systems with an adjacent outdoor area. If the outdoor area makes it easier to clean because of increased urination and defecation on the slats inside, this

could also be an important economic argument for motivating farmers to use outdoor areas as it decreases the heavy workload of cleaning pens.

Pigs' eliminative behaviour can be altered by a few environmental factors. At higher ambient temperatures (≥19 °C), pigs begin seeking cooler areas for resting (Huynh et al., 2005). Over solid floor, pigs start to prefer slatted area for resting as it is cooler (Huynh et al., 2004). Thus, we should aim to keep room temperature below this limit, and rather make warmer microclimates for young pigs that have lower critical temperature. At a higher density (less lying space per pig), pigs eliminate more frequently on solid floor, while they begin to rest more frequently on slatted floor areas (Ocepek and Škorjanc, 2013; Ocepek and Škorjanc, 2016). Reducing animal density in the pens is therefore crucial. We can achieve a dry and a clean pig pen by providing an optimal resting area and area for eliminating. Resting area should consist of solid, insulated floors, closed pen partitions, and feeders located in the corners. Lying areas must be large enough for pigs to rest comfortably during the whole growing-fatting period. By contrast, eliminating area should be a separate area, preferably outdoor, on slatted and wetted floor, bright enough and cooler, with access to drinkers.

Conclusion - important criteria for future housing design

It is beneficial to keep as stable social groups as possible and to design an environment where attractive resources, such as drinkers, feeders, areas for rooting or resting are distributed in a way that makes it difficult to monopolize for a few individuals.

Pigs should be offered more space and substantially more enriched environments including different sources of rooting material at least twice a day and positive challenges (i.e. self-administered rooting material dispenser or other simple problem-solving tasks) in their environment in all stages of production from birth until slaughter. This would lead to less frustration and therefore fewer incidents of redirected, harmful behaviours and rather an increase in positive, social behaviours.

Planning of future housing systems should include greater use of controlled, outdoor arenas. This is a cheaper way of making more stimulating environments, and results in more optimal eliminative habits such as using the inside, slatted area more. This will also reduce the workload regarding cleaning of pens.

Larger groups with a larger total space are socially more stimulating, leads to more locomotion and a higher activity level, and demands less pen equipment and pen dividers. Deep bedding can be removed once or twice a year with a tractor, whereas most small pen systems require substantial effort every day to clean each pen.

To achieve a sustainable pig production in the future, there is a need for substantially more complex pig environments than what we see in most pig production units today. This requires a change is how we value meat, and that the consumers are willing to pay more for good quality meat, where quality also means "quality of life" for these animals. This is also needed to ensure good human health in the future.

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ANTIMICROBIALS

Correlation of tilmicosin pulmonary bioavailability and minimum inhibitory concentration of respiratory pathogens

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Introduction

A way to prove the efficiency of a given antibacterial drug against a microorganism is by conducting pharmacokinetic profile studies after administering the molecule *in vivo*, and comparing the results of these studies with those found in the test for determining the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), performed *in vitro*. For the effectiveness of antimicrobials against respiratory diseases, it is possible to correlate the concentrations found in the lung with the MIC and MBC of the microorganisms of interest. Tilmicosin is a semi-synthetic antimicrobial of the macrolides class, used exclusively in veterinary medicine (1), indicated for the treatment of the complex of respiratory diseases of swine, caused by *Mycoplasma hyopneumoniae, Pasteurella multocida, Haemophilus parasuis* and *Actinobacillus pleuropneumoniae* (2,3,4). An anti-viral effect of tilmicosin was also observed in vitro, in this assay the replication of the PRRS virus reduced by 50% (5). The aim of this study was to correlate the drug concentration in the lung after oral administration with MIC and MBC and to evaluate its effectiveness against the main respiratory pathogens.

Materials and Methods

The Minimum Inhibitory Concentration (MIC) for tilmicosin was tested using bacterial species of great importance in pig farming, and the strains came from Brazilian clinical isolates. MIC data for *Mycoplasma hyopneumoniae* were taken from the literature (3). The test system consisted of 24 young pigs with an approximate body weight of 33 kg (12 males and 12 females), and four animals were used per collection time. Tilmicosin concentrations in the lung tissue were evaluated at 0.5h, 2h, 3h, 4h, 6h and 12h after oral administration by gavage of 20mg/kg/live weight, analyzed by liquid chromatography coupled with mass spectrometry (UPLC/MS/MS).

Results

Tilmicosin is rapidly absorbed and distributed after oral administration to pigs, reaching an average maximum pulmonary concentration above 4 µg/g (Cmax) after 3 hours (Tmax) (Fig. 1). Comparing the pulmonary concentration obtained with the average MIC of each microorganism (Table 1), it is observed that the average concentration of tilmicosin reaches levels higher than the MIC of all evaluated bacteria.

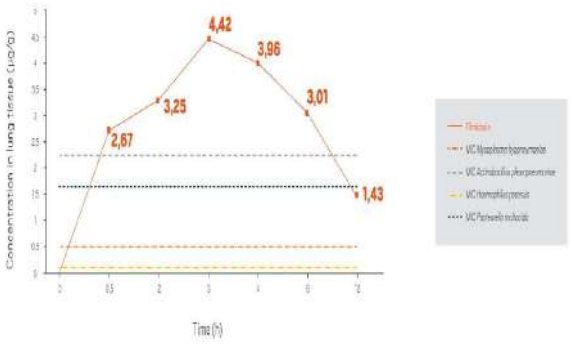

Figure 1. Correlation of the pulmonary concentration of tilmicosin x MIC of the main respiratory pathogens.

Table 2. MIC values of tilmicosin for five strains of *Actinobacillus pleuropneumoniae, Haemophilus parasuis*, *Pasteurella multocida* and one of *Mycoplasma hyopneumoniae.*

Microorganisms	MIC range (µg/mL)	MIC average (µg/mL)
Actinobacillus pleuropneumoniae	1-4	2,2
Haemophilus parasuis	0,06-0,12	0,1
Pasteurella *multocida*	1-2	1,6
Mycoplasma hyopneumoniae	0,5	0,5

Conclusions

The oral administration of tilmicosin to pigs at a dosage of 20 mg/kg/live weight/day reaches tissue levels above the minimum concentration necessary to inhibit the respiratory pathogens of interest in pig farming.

Acknowledgments

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Evaluation of the clinical and reproductive safety of valnemulin in pregnant sows

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Introduction

The efficiency of pig production is related to the use of medicines, which have been used as prophylactic and therapeutic. However, it is necessary to certify that such drug is safe for the target species, yet, in the case of use in pregnant females, it is essential to assess the risks to the offspring. Valnemulin is a semi-synthetic derivative belonging to the group of pleuromutilins, of exclusive use in veterinary medicine (1), indicated for the treatment of the Porcine Respiratory Disease Complex (PRDC), caused by *Mycoplasma hyopneumoniae, Pasteurella multocida, Haemophilus parasuis, Streptococcus suis* and *Actinobacillus pleuropneumoniae* (2, 3, 4). The objective of this study was to evaluate the safety of using the active valnemulin in pregnant sows and its effects on litter.

Materials and Methods

Sixteen pregnant sows of commercial lineage and their progeny (234 piglets) were divided into two groups: the control, with pregnant females that did not receive valnemulin in feed, and the medicated, with pregnant females submitted to a dose of 10 mg of valnemulin per kilogram of body weight administered in feed during seven consecutive days before farrow. The sows were monitored from eight days before farrow to seven days after birth, with blood samples taken for hematological analysis at three moments (before beginning the treatment, one day after beginning and one day after the end of treatment). In addition, the number of piglets born alive, number of piglets born dead and mummified was evaluated. Piglets' weight was measured at three days of age and blood was taken at seven days of age.

Results

Sows and piglets presented normal behaviour and clinical aspect in both groups. Hematological evaluations did not show statistical significant changes in the parameters analyzed between the groups, both in sows and in their respective litters. In addition, when comparing the different collection moments (before, during and after the end of treatment) within the group of medicated sows, no differences were found and the hematological parameters remained similar. The results found were in accordance with the reference values for species (1), corroborating the results of the clinical and behavioral evaluations of the animals in the experiment. The results obtained in the litter evaluations are shown in Fig 1. It is observed that there was no difference between the groups (control and medicated) in the evaluated parameters. The piglets' weight at three days of life (2.035 kg for the control group and 2.030 kg for the medicated group) also did not differ between treatments.

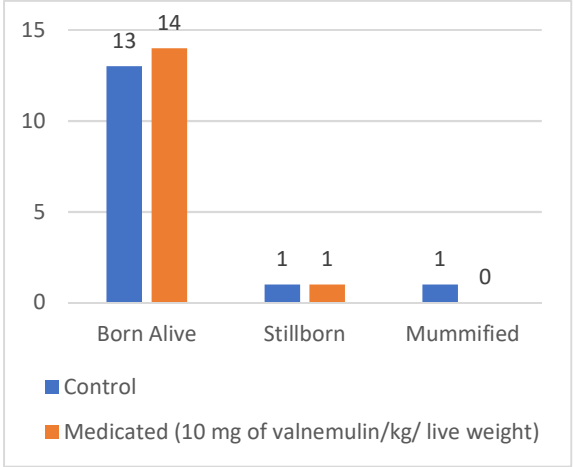

Figure 1. Characteristics of piglets from sows treated with valnemulin in the pre-partum period.

Conclusions

The results obtained through clinical inspections, hematological evaluations and characteristics of the litter demonstrated that the oral administration of valnemulin to sows in the pre-partum period does not cause adverse effects.

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Antibiotic resistance of *Pasteurella multocida* in Spanish swine farms

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Introduction

Antibiotic resistance (AMR) has become one of the major concerns within Public and Animal Health. The cause underneath this problem is the unjustified use of antimicrobial compounds on livestock and medical fields. *Pasteurella multocida* is a facultative anaerobic, non motile Gram-negative bacteria from the upper respiratory tract of pigs, where it usually appears as a commensal organism. Nevertheless, it belongs to Porcine Respiratory Disease Complex (PRDC) and can cause pneumonia and atrophic rhinitis along with other microorganisms as *Bordetella bronchiseptica*. Respiratory diseases related to PRDC are associated to great economic losses and become one of the major issues in swine industry.

This work studies 50 *P.multocida* strains isolated from 110 pneumonic slaughtered pigs. The isolates were examined for capsule biosynthesis, virulence factors and antibiotic resistance associated genes by polymerase chain reaction (PCR). On the other hand, an antimicrobial susceptibility testing (AST) was performed using Sensititre AIM™, with the purpose of implementing a responsible use of antibiotics and looking for alternatives to these treatments.

Materials and Methods

Bacterial isolates were collected from pneumonic pig lungs in slaughterhouses. *P. multocida* was confirmated by extracting DNA and performing a conventional PCR, amplifying a 460 bp product with the primers *KMT1SP6* and *KMT1T7* and electrophoresis in agarose gel. A total of 50 isolates corresponding to *P. multocida* were seen, of which 49 belong to capsular type A (associated with pneumonia in post-weaning piglets) and one to type D (less frequently isolated and associated to atrophic rhinitis in pigs). In order to determine virulence profile 10 virulence-associated genes (*hgbA, nanH, oma87, ompH, pfhA, ptfA, sodA, tbpA, tonB* and *toxA*) were tested by PCR; In adition, we analyzed 8 antibiotic resistance genes: *tetA*, *tetB*, bla_{ROB1}, bla_{TEM}, *ermA, ermC, msrE* and *mphE*. Finally, AST was performed using Sensititre™ Bovine/Porcine BOPO6F Vet AST Plates, which contained eighteen different antibiotics.

Results

All *P. multocida* strains amplified *nanH* and *oma87* virulence genes, while *hgbA, ompH*, *ptfA*, *sodA* and *tonB* were present in more than 90% of the isolates; and *toxA* and *pfhA* in 38% and 36% of the isolates, respectively. In contrast, only one of the isolates showed the *tbpA* gene. Concerning to antimicrobial resistance genes, *ermC* was the most prevalent one, being present in 40% of the isolates, while *tetB, blaROB1* and *msE* were detected in the opposite 38%, 28% and 24% of the bacteria, respectively. The least prevalent antimicrobial resistance genes were *ermA*, *tetA* and *mphE,* found in 16%, 12% and 2% of the isolates, respectively.

AST results confirmed that nearly all *P. multocida* isolates were resistant to lincosamides. Nevertheless, they exhibited a high susceptibility to aminoglycosides, β-lactams, phenicols and quinolones, with more than 90% of the isolates being considered sensitive against these antibiotics.

Conclusions and Discussion

This study has shown data about the antibiotic resistance and virulence gene profile of swine *P. multocida* recovered in Spain. Antimicrobial resistance gene results differed for those encoding resistance to macrolides, for which 20 of the 50 isolates exhibited the *ermC* gene, whereas only one of them presented the *mphE* one. These ranges were also observed for genes encoding resistance to β-lactams (14 isolates being positive for bla_{ROB1}, but only 4 for bla_{TEM}) and for those encoding resistance to tetracyclines (19 isolates amplified *tetB;* while 6 amplified *tetA*). Virulence-associated genes were more present in *P. multocida* than antimicrobial resistance genes (with the exception of *tbpA (*which was absent in those tested isolates), *pfhA* and *toxA,* the remaining seven virulence factor genes where present in more than 90% of the isolates.

In regards to antimicrobial susceptibility testing, enrofloxacin, ceftiofur, florfenicol, neomicin and gentamicin were highly active *in vitro* against these isolates and, consequently, they remain useful for treatment of swine pneumonia caused by this pathogen.

However, *P. multocida* has developed resistance to other antibiotics. The key to stop this emerging issue should be to focus in developing alternatives to antimicrobials. Nevertheless, a good prescription, use and management of antimicrobial compounds along with a good farm management and an efficient use of disinfectants could represent a useful aid to stopping this abusive use of antibiotics.

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Effect of the production system in the antimicrobial resistance in *Staphylococcus* spp. isolates recovered from Spanish pig herds

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Introduction

Antimicrobial resistance (AMR) can be considered as a worldwide public health threat, being antimicrobial use in food-producing animals an important contributor (1). Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) is one of the main zoonotic bacteria in swine production (2), but other *Staphylococcus* species are being considered a major nosocomial burden and play also an important role as reservoir of AMR genes for *S. aureus* (3). Despite most of the recent epidemiological studies focus on MRSA, the prevalence of AMR in *Staphylococcus* in pigs and farm environments, beyond MRSA, is not very well established. Thus, the objective of this study was to evaluate the prevalence of AMR on intensive and extensive Spanish pig farms.

Materials and Methods

A total of 37 Spanish swine farms were sampled and divided into intensive (18 herds) and extensive (19 herds) based on their production system.

AMR for *Staphylococcus* spp. isolates was evaluated by the broth microdilution method with EUST Sensititre plates (TREK Diagnostic Systems, East Grinstead, UK) following EUCAST guidelines.

Percentage of resistance among production systems was compared using Chi-square test at α=0.05 (R Project, version 3.6.1)

Results

Thirteen different species were identified in the 84 *Staphylococcus* isolates recovered in the study, being *S. haemolyticus* (25), *S. chromogenes* (15) and *S. hyicus* (14) the predominant ones.

AMR was predominant for clindamycin (61.9%), tetracycline (59.52%), tiamulin (58.33%) and trimethoprim (52.69%). No resistant isolates were found for vancomycin.

AMR was significantly higher on intensive swine farms as compared to extensive production systems for clindamycin ($p < 0.001$), tiamulin ($p < 0.001$), quinupristin/dalfopristin ($p < 0.001$), erythromycin ($p < 0.001$), tetracycline ($p = 0.001$), penicillin ($p = 0.005$), ciprofloxacin ($p = 0.012$) and chloramphenicol ($p = 0.036$), and also for the multidrug-resistant phenotype ($p < 0.001$).

Discussion and conclusions

Differences in prevalence and antimicrobial resistant patterns in *Staphylococcus* spp. isolated in our study compared to previous studies in Belgian (4) or German (5) pigs might be associated to the species detected, the type of sample collected or the antimicrobial use on their farms. It is remarkable that in China, Guo et al. (6) observed very high resistance prevalences to most of the antimicrobials evaluated, suggesting that these findings might be related to the abuse of antimicrobial agents in livestock animals.

In this sense, a minor traditional use of antimicrobials in extensive Iberian pigs due to low densities of animals might be an important reason for the differences found in this study among intensive and extensive farms.

In conclusion, the intensive production system seems to have an impact on the AMR phenotype of *Staphylococcus* isolated on swine farms. Thus, interventions in intensive pig herds need to be implemented in order to reduce antimicrobial use and, hence, resistance prevalences.

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Probiotic *Bacillus licheniformis* supports growth performance of fattening pigs under *Clostridium perfringens* stress

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Introduction

As the effort towards antimicrobial free production continues, alternative feed additives that can support growth performance and disease mitigation have gained interest. Probiotics are a good example, as these viable microorganisms confer health benefits to the host, if administered in adequate amounts (FAO/WHO, 2002). To test the efficacy of probiotic *Bacillus licheniformis* (DSM 28710) in terms of supporting growth performance, a trial was set up on a commercial fattening farm in northern Jalisco (Mexico).

Material and Methods

Animals were divided over two treatments, using 208 fattening pigs (average weight at the start of the trial: 29.5 kg) in total. Each treatment had 2 replicates. The trial was carried out during a high *Clostridium perfringens* challenge period, as determined by the historical records of the farm. Animals were supplemented with either treatment for 70 days, starting when they arrived on-farm. The first treatment was a BMD® group, fed a commercial basal diet supplemented with 300 g BMD® (bacitracin methylene disalicylate) / mton of feed. The second treatment, a B-Act® group, was fed the same commercial basal diet (no BMD®) but supplemented with 500 g B-Act®/mton of feed (1.6 x 10^{12} CFU *Bacillus licheniformis*/mton of feed). B-Act® is a probiotic feed additive containing viable spores of *Bacillus licheniformis* DSM 28710. Average daily gain (ADG), average daily feed intake (ADFI) and feed conversation ratio (FCR) were evaluated on day 40 and 70, whilst mortality was recorded daily.

Results

Throughout the trial, there were no statistically significant differences between the two treatments, for none of the parameters evaluated (Table 1, Table 2, $P<0.05$).

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Table 1. Growth performance parameters for the two treatments, from day 0 to 40

Day 0 - 40		
Parameter	**BMD®**	**B-Act®**
ADG (kg)	0.750	0.779
FI (kg)	1.612	1.665
FCR	2.134	2.146
Mortality (%)	0	0

Table 2: Growth performance parameters for the two treatments, from day 0 to 70

Day 0-70		
Parameter	**BMD®**	**B-Act®**
ADG (kg)	0.856	0.928
FI (kg)	1.767	1.871
FCR	2.063	2.016
Mortality (%)	7.69	7.69

Discussion and conclusion

In this trial, B-Act® was as efficacious as BMD® in terms of maintaining technical growth performance during high *Clostridium perfringens* stress. As such, B-Act® provides an interesting alternative to support profitable animal production on-farm, whilst reducing the use of antimicrobials. This ties in with the wider global effort of reducing the prophylactic use of antimicrobials, confirming the contribution probiotics can make.

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Pharmacokinetic behavior of a patented florfenicol formulation for use in drinking water

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Introduction

Florfenicol is a lipophilic molecule showing a fast intestinal absorption and excellent tissue penetration (1). The pharmacokinetic behavior was studied by a continuous administration of a patented florfenicol formulation (Amphen® 200 mg/ g water soluble granules - Huvepharma®) in the drinking water of pigs in order to evaluate the molecule's efficacy for the treatment of several important respiratory pathogens.

Materials and Methods

6 pigs (53 to 61 kg bodyweight) were continuously treated via the drinking water at a daily dosage of 5 mg florfenicol/ kg bodyweight for 5 consecutive days. Blood samples were taken on day 1 (0, 0.5, 1, 2, 4, 8, 12, 24 h) and day 5 (96, 98, 100, 104, 108, 120 h) to determine the plasma florfenicol concentration by High Performance Liquid Chromatography. The mean florfenicol concentration at each time point was calculated and compared with the Minimal Inhibitory Concentration to inhibit growth of 90 % (MIC_{90}) of *A. pleuropneumoniae, H. parasuis* and *P. multocida* strains. The florfenicol MIC_{90} value of these 3 bacteria is 0.5 µg/ ml (2), indicating an excellent in vitro susceptibility.

Results

High plasma florfenicol concentrations were noted starting from 0.5 h after the start of the treatment and remained largely above the MIC_{90} for all 3 pathogens for the whole duration of the treatment. Maximum plasma concentrations of 1.6 and 1.9 µg/ ml were determined on day 1 (fig.1) and 5 (fig.2) respectively.

Figure1. Mean plasma florfenicol concentrations day 1

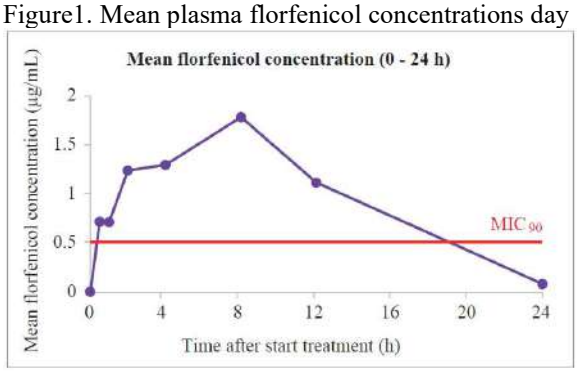
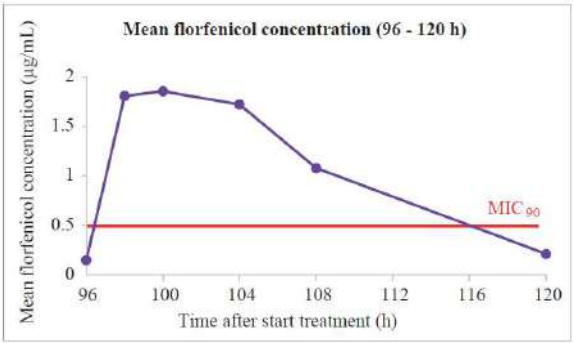


Figure 2. Mean plasma florfenicol concentrations day 5



Conclusions and Discussion

Florfenicol plasma concentrations far above the MIC_{90} values for important respiratory bacteria were found immediately after the start of the administration of Amphen® via the drinking water at a daily dose of 5 mg/ kg bodyweight. The excellent tissue penetration of florfenicol and the high susceptibility of a broad spectrum of respiratory pathogens results in a fast and optimal clinical recovery of lung infections.

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Reduced usage of Highest Priority Critically Important Antimicrobials in pigs within the SuisSano program in Switzerland

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Introduction

In order to improve transparency and to monitor antimicrobial usage in pig production in Switzerland, the SuisSano program was started in 2015. One important goal of this program was to reduce the usage of Highest Priority Critically Important Antimicrobials (HPCIAs) in participating pig farms. For this reason, treatments with HPCIAs were multiplicated with factor four, when reporting antimicrobial usage to the farmers within the SuisSano program (1-3). Since April 2016 the use of HPCIAs is restricted by legal regulations in Switzerland. Defined Course Doses have been established for treatments of pigs in Switzerland in order to describe antimicrobial consumption (2,3).
The aim of the study was to investigate the impact of both legal regulations and private initiatives in reducing the usage of HPCIAs

Materials and Methods

The number of study farms was 312 in 2015, 483 in 2016 and 598 in 2017. All veterinary prescriptions were assigned to four age groups: suckling piglets, weaned piglets, fattening pigs and sows. Antibiotic usage in the study farms was calculated based on Defined Course Doses (DCDCH) per animal per year and analysed by antibiotic classes for the years 2015, 2016 and 2017.

Results

The relative usage of HPCIAs in all age groups decreased from 25% in 2015 to 10% in 2017. In sows the relative usage decreased from 17% in 2015 to 2% in 2017 (table 1), in piglets from 20% to 5%, in weaners from 42% to 26% and in fattening pigs from 8% to 3%.

Table 1.Usage of HPCIAs (number of DCDch) and number of animals (n) in the study in 2015 and 2017 per age category

	2015		**2017**	
	n	HPCIAs	n	HPCIAs
sows	16.172	2.732	25.358	855
suckling piglets	447.097	65.812	690.817	20.012
weaned piglets	391.739	46.094	599.313	18.059
fattening pigs	308.492	4.981	403.861	2.424
Total	**1.163.500**	**119.619**	**1.719.349**	**41.350**

Conclusions and Discussion

The reduced usage of HPCIAs described in this study was most likely induced by legal regulations and the impact of the multiplication factor of such treatments within the SuisSano programme. Because of the voluntary character of the SuisSano program participating farmers may be more motivated than others to reduce the usage of HPCIAs on their farms.

Acknowledgements

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Strategic use and value of spray dried plasma in grow-finish pig feed instead of growth promoting antibiotics on health and performance under commercial conditions in Brazil

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Introduction

Spray-dried plasma (SDP) is a highly digestible, high-protein feed ingredient that is widely used in feed programs for weaned pigs. The benefits of SDP on growth performance, gut function, and immune parameters are well documented (1, 2). The objective of this work was to evaluate the effects of strategic use of spray-dried plasma instead of antibiotic growth promoters (AGP) in feed on health and performance of grow-finish pigs under commercial conditions of Brazil to determine if SDP can reduce reliance on antibiotics.

Material and Methods

A total of 240 grower pigs (65 d of age) were randomly assigned to either a control or SDP feed treatment group. At housing, pigs were divided into 6 pens of castrated males and 4 pens of females, with 10 reps per treatment. Both groups used the same prophylactic antibiotic pulse program (Avilamycin, grower 1) and (Florfenicol, finisher 1). The SDP group used 1.5% or 0.75% SDP formulated in the respective grower 1 and finisher 2 diets and did not use the growth promoters (Flavomycin, grower 1, 2, finisher 2, 3; Tylosin finisher 2, 3; yeast cell wall product in all diets) used in the control feed program. Animals were kept at the farm until slaughter at 146 d of age. Individual body weights were recorded at 65, 105 and 146 days of age, and pneumonia prevalence index (IPP) of lungs from 100 pigs per treatment was recorded after slaughter.

Results and discussion:

Treatment effects on average body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), feed conversion (F/G) and income over feed cost (IOFC) are shown in table 1. Between 65 and 105 d of age the SDP group had better ADG and F/G and higher BW at d 105 ($P<0.05$). ADG, ADFI and F/G did not differ between groups during 105-164 d of age (data not shown). Over the entire study F/G was better ($P<0.10$) for the SDP group. The IPP index was 0.30 and 0.45, respectively for control and SDP treatments. Both groups were classified as mild pneumonia (< 0.50 IPP). Only 3 pigs died during the study; 2 Control pigs and 1 SDP pig. The SDP group returned the highest IOFC.

These results agree with a different study that also reported better performance and income using SDP to replace AGP in feed for grow-finish pigs (3). In both cases SDP helped to reduce the reliance on growth promoting antibiotics.

Table 1: Performance and economic results.

	Treatment Groups	
Variable	Control	SDP
Initial BW, kg	25.90	25.90
ADG kg, d 65-105	0.88^{b}	0.93^{a}
ADFI kg, d 65-105	1.87	1.86
F/G, d 65-105	2.11^{a}	2.00^{b}
BW kg, d 105	62.13^{b}	64.09^{a}
ADG kg, d 65-146	1.04	1.05
ADFI kg, d 65-146	2.30	2.28
F/G, d 65-146	2.21^{x}	2.17^{y}
Final BW kg, d 146	109.8	110.7
IOFC, US$	$102.27	$103.00

Means with uncommon superscript differ; ^{a,b} ($P< 0.05$); ^{x,y} ($P< 0.10$). IOFC = Income over feed cost. Hog price = US$ 1.27/ kg live weight.

Conclusions:

Under the conditions of this study, SDP inclusion in grower 1 and finisher 2 diets improved performance and IOFC by $0.73 USD/pig, thus offering a novel approach in accordance with new antibiotic and environmental regulations to reduce reliance on antibiotics.

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Case Report: Reduction of Antimicrobial Use in Sow to Finish Farm in Brazil

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Introduction

Antimicrobial resistance is a worldwide problem, which impacts human and animal health. Misuse and overuse of antimicrobials in the animal industry, including swine production, is an important part of the issue (1,2). This case report analyses the ability of an integrated approach, including nutritional, farm management and health strategies in reducing the use of antimicrobials in a commercial farm, maintaining animal performance.

Materials and Methods

A longitudinal study was performed on a commercial sow to finish farm with 500 sows in Brazil from Jan 2017 - Nov 2019. Farm was negative to *Mycoplasma hyopneumoniae* and positive to SIV, *Streptococcus suis, Haemophilus parasuis, Salmonella enterica*, *Lawsonia intracellularis, Escherichia coli* (toxigenic strain) and *Clostridium perfringens* type *A*. During 3 years, different interventions (Table 1) were undertaken and afterwards antimicrobial (AM) use (via feed, water and parenteral) was monitored by measuring mg AM/kg body weight (BW), quantification of active compound used preventively and period of use of AM (days / slaughter age). Annual average performance and health parameters were compared over 3 years.

Table 1. Interventions or changes made

Feed
Nutritional: optimize sow diet to increase milk production
Functional ingredients: plasma, probiotics, essential oils, organic acids
Farm
Biosecurity: checklists, protocols
Management: less cross-fostering, increase in weaning age
Environmental: optimization climate, slatted flooring
Drinking water: reduce pH using organic acids
Health
Diagnostics: urinalysis, feces and tissue for pathogen load in farrowing and nursery
Health evaluation in slaughterhouse
Vaccination: update protocols

Results

Performance was similar over 3 years (Table 2). From 2017 to 2019, AM use decreased in number of active components used from 8 to 2, amount of usage days from 85.6% to 18.6% of growing days. The use of AM/kg BW was reduced from 521.4 mg in 2017 to 30.2 mg in 2019 (Table 3).

Table 2. Performance (annual average).

Parameters	**2017**	**2018**	**2019***
Farrowing rate (%)	93.6	92.9	91.4
Weaned piglets/sow/year	29.5	30.4	30.9
ADG nursery (kg/d)	0.413	0.402	0.392
Mortality nursery (%)	1.03	1.14	1.08
ADG finisher (kg/d)	0.882	0.906	0.927
Mortality grower-finisher (%)	0.82	0.99	1.87**

*2019: data from January to November **Influenza outbreak in September

Table 3. Antimicrobial usage (annual average).

Parameters	**2017**	**2018**	**2019**
Active compounds preventively (n)	8	4	2
Usage period (days/slaughter age)	137/160	40/156	29/156
Usage period (% of days)	85.6%	25.6%	18.6%
mg AM/kg BW	521.4	133.7	30.2

Conclusion and Discussion

Results indicate an integrated approach, including feed, farm and health management, can support a reduction in use of antimicrobials in swine production, even in traditional farms with challenges, without negatively impacting technical parameters.

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Effects of synergistic blend of organic acids on performance of grow-finish pigs and antibiotic resistance of *Escherichia coli*

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Introduction

In the post AGP era, alternatives like the synergistic blend of feed additives (FA) have been regarded as a potential antibiotic replacement because of their health-protecting and growth-promoting properties (1). Synergistic FA has a broad antimicrobial effect and support a functional gut necessary to enhance animal performance. The study aims to validate the efficacy of a synergistic blend of FA on performance and its effect on antimicrobial resistance of *E. coli* isolated from faeces of grow-finish pigs.

Materials and Methods

This study was conducted on a commercial farm in Vietnam with 312 grower pigs (26.5 ± 0.92 kg) allocated to one of three treatments including negative control (NC, a basal diet), AGP (NC+Colistin 20 ppm/t feed) and synergistic blend of short- and medium-chain fatty acids (SGG, NC+2 kg SGG/t feed). The zootechnical performance including body weight gain (ADG), feed consumption (ADFI) and feed conversion ratio (FCR) were monitored for a 90-day feeding period. On the last day of the grower phase (d45), fresh faecal samples (100 g) were taken from 2 pigs randomly selected per pen, which were pooled and sub-sampled (100 g). Faecal samples were checked for the prevalence of *E. coli* and subjected to an antibiogram test. The isolates were tested for susceptibility against Colistin (10 µg), Amoxicillin/Colistin(10/10µg), Amoxicillin/clavulanic acid (20/10µg), Cefotaxime (30µg), Ceftiofur (30µg), Ciprofloxacin (5µg), Norfloxacin (10µg) on Mueller-Hinton agar plates by disc diffusion method as per the criteria of the National Committee for Clinical Laboratory Standards (1).

Results

SGG elicited a similar effect as AGP on feed cost and on all growth parameters measured (P>0.05). In relation to NC, SGG significantly improved final body weight (3.4 kg or 3.6%), ADG (+39 g or 5.3%) and FCR (−20.2 points or 7.4%) of pigs (Table 1). No significant differences were seen in feed intake and mortality (data not shown). In addition, the feeding of SGG reduced feed cost (−0.053 USD) per kilogram body weight gain. The *E. coli* isolates in the study were multi-drug resistant. However, when SGG was added in the diet, the resistance of *E. coli* to Amoxicillin/clavulanic acid, Cefotaxime, Ceftiofur and Norfloxacin was reduced (data not shown); whereas the susceptibility of *E. coli* to Amoxicillin-clavulanic, Cefotaxime, Ceftiofur, Ciprofloxacin and Nofloxacin were increased compared to AGP (P<0.05, Table 2).

Table 1. Effects of treatment on the performance of grow-finish pigs from day 1-90 of the fattening period

	Final BW, kg	ADG, g	FCR
NC	92.49^{b}	741.19^{b}	2.736^{a}
AGP	94.99^{a}	769.67^{a}	2.609^{ab}
SGG	95.84^{a}	780.23^{a}	2.534^{b}
SEM	1.694	18.072	0.081
P-value	<.0001	<.0001	0.0007

^{a,b}Means within a column having a different letter differ significantly (P<0.05).

Table 2. Susceptibility to selected antibiotics of *E. coli* isolated in feces of grow-finish pigs

Antibiotics	NC	AGP	SGG	P-value
Amoxicillin	0	0	0	-
Amox-Colistin	0	0	0	-
Amox-clavulanic	12.5^{a}	0^{b}	14.3^{a}	0.001
Ceftiofur	0^{b}	12.5^{a}	14.3^{a}	0.001
Ciprofloxacin	12.5^{a}	0^{b}	14.3^{a}	0.001
Norfloxacin	0^{b}	0b	14.3a	<0.001
Cefotaxime	37.5^{b}	12.5^{c}	57.1^{a}	<0.0001

^{a,b}Means within a row having a different letter differ significantly (P<0.05).

Conclusion

The findings of this study suggest that SGG is a cost-effective product with the same efficacy as AGP in promoting the growth and economic performance of fattening pigs. Antibiogram analysis shows that *E. coli* isolates are multi-drug resistant, proving the issue and urgency in swine production to find alternatives to antibiotics. SGG can be used as a replacement for AGP to secure animal performance without developing resistance to selected antibiotics.

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Efficacy of Nuflor premix in Chinese swine farms

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Introduction

Glasser's disease is caused by Haemophilus parasuis (HPS), a commensal organism of the upper respiratory tract of swine that causes severe systemic disease characterized by fibrinous polyserositis, arthritis, and meningitis. Young animals (4–8 wks old) are primarily affected, although sporadic disease can be seen in adults (eg, introduction of a naive adult to a healthy herd). Porcine pleuropneumonia is caused by Actinobacillus pleuropneumoniae (App) affecting animals of all ages, however it is more frequently found among pigs at 12-16 weeks of age. Both pathogens can cause severe consolidated pleurisy, which can result in reduced growth rate and carcass condemnation at slaughter. Nuflor premix (2% florfenicol) is an in-feed antibiotic labelled for the treatment of HPS or APP. The objective of the work presented here was to determine the efficacy of Nuflor premix in Chinese swine farms.

Materials and Methods

Two individual experiments were conducted to demonstrate the efficacy of Nuflor premix.

Experiment 1- A farm of 2,500 sows in Jiangsu Province, China. Farm was PRRS unstable with nursery pigs showing peak clinical lesions of HPS at 40-45 days of age and 10% mortality. 922 pigs at 35-40 days of age were distributed randomly into 2 groups. Group A with 454 animals that were non medicated and Group B with 468 animals with feed medicated with Nuflor premix 2kg/ton for 7 days. The morbidity and mortality were recorded every day during the nursery period.

Experiment 2- Three different farms in Jiangsu Province and Anhui Province, China with a total of 11,000 sows across the three farms. APP was isolated through bacterial culture from grower pig mortalities. Affected grower pigs (Anhui 856, Jiangsu 1540) were treated with Nuflor premix medicated feed (4 Kg/T) for 7 days. The feed medication commenced at the peak time of morbidity and mortality on the farms. The following performance parameters were analysed.

1. Cure rate: The proportion of test pigs that returned to normal alertness, appetite, stool, and body temperature after 3 days of medication.
2. Improvement rate: The proportion of test pigs that improved significantly in terms of alertness, appetite, stool and body temperature after 3 days.
3. Ineffective rate: The proportion of pigs that did not respond or worsened after 3 days in terms of alertness, appetite, stool and body temperature.

Results

Table1. The record of morbidity and mortality in nursery period.

Group	Number of pigs	Morbidity	Mortality
A	454	32%	10%
B	468	13%	4%

Table 2. The record of cure rate, improvement rate and ineffective rate.

Area	Morbidity (APP)	1	2	3
Anhui	28%	72%	12%	17%
Jiangsu	19%	75%	13%	12%
Ave	22%	73%	13%	14%

1: Cure rate; 2: Improvement rate; 3: Ineffective rate.

Conclusions and Discussion

The results in Experiment 1 indicate that administration of Nuflor premix at a dose of 2kg/ton for 7 days can significantly reduce the morbidity and mortality caused by HPS in the nursery period.

The results in Experiment 2 showed that Nuflor premix at a dose of 4kg/ton for 7 days was highly effective in curing affected pigs suffering from APP in this production system.

In vitro activity of tildipirosin against clinical isolates of *Glaesserella parasuis*

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Introduction

Glässer disease (GD) is one of the most important and worldwide spread bacterial diseases of swine. The disease is caused by *Glaesserella parasuis* and is characterized by pneumonia, polyarthritis, meningitis and polyserosites. Commercial vaccines are globally available but with little or no protection against heterologous serovars (SV) usually found within the same geographic region (1). Thus, outbreaks of GD need to be rationally restrained by antibiotics treatment. In this sense, here we evaluated the susceptibility of one hundred Brazilian clinical isolates of *G. parasuis* to tildipirosin, an antimicrobial drug solely used in veterinary medicine.

Materials and Methods

One hundred clinical isolates belonging to our *G. parasuis* collection were included in this study. The bacteria were isolated from January 2013 up to June 2018 from pigs with clinical signs and macroscopic lesions consistent with GD, and were stored at -80ºC. Amongst the isolates, 58 were from systemic sites of infection (peritoneum, joints, pericardium and cerebrum) and 42 were from lungs. All isolates included in this study were from different farms and from states with high pig production rates; in addition, after culture isolation, all samples were molecularly typified (1) . The susceptibility test was carried out by the microdilution assay (96 well plates, TPP, Swiss) according to the recommendation of the CLSI (2013) for *Actinobacillus pleuropneumoniae*, using PPLO media supplemented with 75 µg/mL of nicotinamide adenine dinucleotide and 2.5 mg/mL of glucose (Sigma-Aldrich, Brazil). The Tildipirosin pure powder was supplied by Merck Sharp & Dohme (Brazil) and serially diluted in duplicates from 256 to 0.032 µg/mL in PPLO media. Then, *G. parasuis* (5×10^4 bacteria) was added to each well and incubated at 37ºC and 5% CO_2 for 24 h, as previously described (2). The lower tildipirosin concentration able to inhibit bacteria growth was considered the minimal inhibitory concentration (MIC).

Results

The MIC distribution of tildipirosin against *G. parasuis* varied from 0.03 to 16 µg/mL, with three peak values, observed at 0.03, 0.06 and 0.25 µg/mL. The distribution ratios of the 3 mean peaks of MIC were 30% for 0.03 µg/mL, 15% for 0.06 µg/mL and 14% for 0.25 µg/mL (Fig. 1A). The MIC_{50} and MIC_{90} were 0.06 and 4 µg/mL, respectively. All isolates with a MIC higher than 4 µg/mL (10%) were isolated from 2015 forward, only one year after tildipirosin introduction into Brazilian pig farms. The susceptibility pattern of systemic and pulmonary isolates was similar (Fig.1B). Considering the relationship between the maximal concentration of the drug in blood serum (C_{max}) and the *in vitro* MIC, the therapeutic concentration of the tildipirosin commercial drug (Zuprevo® MSD, 4 mg/Kg) administered by the intramuscular route, as recommended by the manufacturer, would render a pulmonary epithelial lining fluid (PELF) C_{max} of 4.06 ± 0.65 µg/mL at 5.33 ± 2.37 hours after injection (3). Considering this, 90% of isolates evaluated in this study could be effectively treated by the therapeutic dose.

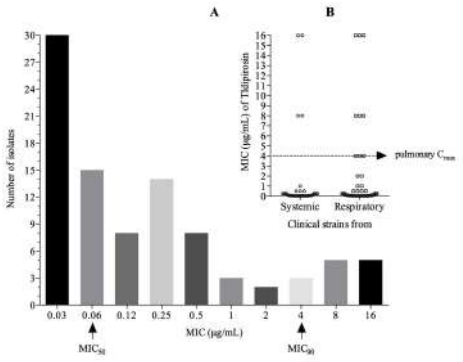

Figure 1. MIC distributions of tildipirosin against *G. parasuis*. **A**: Cumulative isolates *per* MIC and values of MIC_{50} and MIC_{90}. **B**: MIC distributions of systemic and respiratory isolates. PELF C_{max} (3) is represented by dashed arrow.

Conclusions and Discussion

Our results indicate that tildipirosin is highly recommended for treating clinical cases of GD; however, because we have detected isolates likely resistant to tildipirosin (10 isolates had MIC > 4 µg/mL), the MIC assay should be periodically used to access the therapeutic concentration to be used and to monitor the evolution of resistant isolates of *G. parasuis* to this novel drug.

Acknowledgments

MSD kindly supplied the tildipirosin pure powder.

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Tylosin used in a high dose for a short period in *Lawsonia intracellularis* naturally infected pigs improves pig performance and reduces agent fecal excretion

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Introduction

Proliferative enteropathy (PE) caused by *Lawsonia intracellularis* (LI), is one of the enteric diseases that most affect the pig industry (1,3,4). Despite, the absence of clinical signs, even in their subclinical form can directly affect average daily weight gain (ADG) with a reduction up to 37% and an increase of 27% in feed conversion ratio (FCR) (2,3). Tylosin is used worldwide to control the disease, but usually low doses that have an effect of growth promotor are used. Thus, the aim of this study was to test if an in-feed tylosin treatment using a higher dose for a shorter period improves pig performance and reduces fecal excretion of LI.

Materials and Methods

We performed a non-blinded randomized controlled clinical trial in a commercial pig farm located in Midwest Brazil. The study involved 300 pigs with 76 days of age (same commercial lineage). The animals were randomly assigned to the experimental group (n=150) and to the control group (n=150). Both of them consisted of five pens with 30 pigs each. The experimental group received in-feed tylosin at a dose of 10 mg/kg BW for 14 days. The control group received feed without tylosin. After the 14 days, both groups were drug-free feed for more seven days.

We obtained ADG by weighing animals in each pen, as an experimental unit, for three times: one day before treatment (day 0), one day after the end of the treatment (day 15th) and eight days after the end (day 22nd). We also calculated FCR by feed intake per pen divided by weight gain during the 14 days of treatment and up to seven days after its end (day 21st).

To the quantitative analysis of LI excretion, we collected random fecal samples from 10 pigs from each pen on days 0, 15th and 22nd. These samples were quantified by qPCR, as previously described (5).

The comparison between the groups was performed using the Kruskal-Wallis test with two-way discrimination using the Mann-Whitney's U test. Values of $p<0.05$ were considered statistically significant.

Results

On day 15th the experimental group showed a higher ADG - about 14% more - than the control group during the treatment period (experimental: 631g *vs*. control: 545g; p=0.047). Otherwise, FCR was not statistically different between groups (experimental: 2.24kg *vs*. control: 2.51; p=0.076).

The mean value of LI excretion was similar on day 0 and on day 15th. However, on day 22nd the treatment group presented a significant reduction compared with the control (Figure 1).

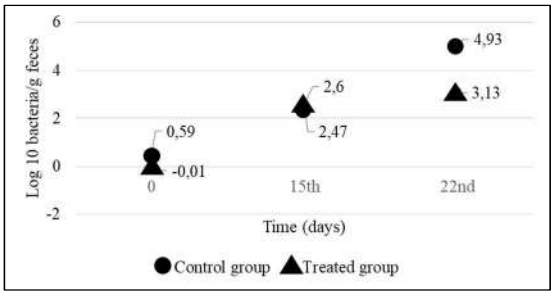

Figure 1. Mean values of excretion of *L. intracellularis* during days 0, 15th and 22nd of fecal samples collection.

Conclusions and Discussion

This study showed that tylosin at the dose of 10 mg/kg BW for 14 days is an applicable alternative to the low-doses of this antibiotic used as a growth promoter. This therapeutical dose of tylosin used for a shorter period was able to improve finishing pig performance and to reduce LI fecal excretion, without the concern of selecting antibiotic-resistant bacteria – that can be a risk to animal and public health.

The mean level of LI excretion presented by the control group is indicative of the presence of intestinal lesions related to PE. Meanwhile, the mean level presented by the treatment group suggests these pigs did not present such lesions (6). Although a low amount of LI in feces could be able for transmission, the reduction of the agent excretion level probably diminishes the environmental contamination in the long-term between batches.

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Purple garlic powder as nutritional additive optimizes intestinal health at the post-weaning stage in commercial pig farms

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Introduction

The weaning period is a critical phase for piglets due to intestinal problems that evolve into diarrhea, increased mortality and productivity lose. Therapeutic doses of ZnO, together with antibiotic treatments, have been widely used to tackle this problem (1). Nevertheless, due to the policy requirements to reduce the dose of ZnO used in piglet diets in the European Union (EU) (2), alternatives to these methods to avoid digestive disorders at critical times such as the post-weaning period are urgently needed.

The objective of the present study was to determine if purple garlic powder improves intestinal health by enhancing the composition of gut microbiota.

Materials and Methods

Escherichia coli, *Enterobacteriaceae* and *Lactobacillus* spp. were quantified in four homogenous groups of weaned pigs 10 weeks old. The groups were fed two different concentrations of purple garlic powder (Table 1). A positive control receiving ZnO (3.1 kg/t) and a negative control (no additives) were included.

The content of the ileocecal valve was sampled and selective culture mediums (Mc Conkey for *Enterobacteriaceae*, RAPID'E.coli 2 for *E. coli* and Rogosa Agar for *Lactobacillus* spp.) were used for the isolation of microorganisms. *Salmonella* spp. was isolated according to ISO 6579.

Data were analyzed by a SPSS one-way ANOVA model, specifying the treatment group as fixed factor. Adjusted means were used to compare for the effects of the different treatments by Tukey's post-hoc test.

Results

Results are shown in Table 1. Both garlic treatments had a statistically significant higher count of *Enterobacteriaceae* compared to the not treated group, and there was not significant difference with the ZnO group. Garlic groups did not show any significant difference with the control groups for *E. coli* and *Lactobacillus* spp. (Table 1). *Salmonella* spp. was not detected in any sample.

Conclusions and Discussion

The results suggested that purple garlic powder has a stabilizing effect on the intestinal microflora, maintaining the diversity of coliforms and preventing the proliferation of opportunistic pathogenic microorganisms such as *Salmonella* spp. (4). These results are in line with bibliography (3).

Their effect was beneficial comparing with the absence of treatment, as the amount of *Enterobacteriaceae* present in the gut was within normal limits, similarly to the ZnO treated group (Table 1).

Table 1. Results of the Tukey's post-hoc to the one-way ANOVA test for the studied microorganisms.

		Mean UFC/g (Log10)		
GROUP[1]	N	*Entero-bacteriaceae*	*E. coli*	*Lacto bacillus spp.*
No treatment	6	3.19^{a}	4.60^{a}	7.21^{a}
Garlic 1%	8	6.02^{bc}	5.78^{ab}	7.79^{a}
Garlic 2%	8	5.95^{bc}	5.68^{ab}	7.28^{a}
ZnO	6	7.28^{c}	7.07^{b}	7.97^{a}

[1]Superscripts (a, b, c) indicate statistically significant differences for the quantified microorganism ($p \leq 0.05$)

This fact reduces the risk of presenting diarrea during the post weaning period. This effect was beneficial comparing to the use of ZnO, which increased the levels of *E. coli*. These results suggest that the use of purple garlic powder at a concentration of 1% in feed is a valid alternative to ZnO.

Further research is needed to define other parameters affected by the use of purple garlic powder as a substitute for ZnO, such as productive, sanitary and wellfare parameters.

Acknowledgments

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Optimizing dosing regimens of benzylpenicillin in an experimental model of *Actinobacillus pleuropneumoniae* infection in pigs

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Introduction

Actinobacillus pleuropneumoniae (APP) cause serious respiratory infections in pigs. According to Swedish guidelines, benzylpenicillin (PC) is recommended for treating acute APP infections (1) but the approved dose of 20 mg/kg at 24-hour intervals has been demonstrated ineffective (2). Relatively high (0.25-0.5 mg/L) wild type minimum inhibitory concentrations (MIC) for APP (3) and large individual variability in PC plasma concentration has resulted in inadequate exposure above MIC which may explain the ineffective treatments (4). The objective of this study was therefore to investigate how treatments of APP infections with procaine PC can be improved.

Materials and Methods

Five groups of six 9- to 11-week-old specific pathogen-free (SPF) pigs were housed in separate rooms with separate ventilation and manure handling systems at SVA. Pigs were inoculated intranasally with APP serotype 2 (10^{11} cfu) one week after arrival. One group served as an untreated control (NT), while intramuscular treatments (im) for three days with procaine PC at different dosages were initiated in the other groups when clinical signs were seen (Table 1). Two formulations of PC available on the Swedish market were used, an aqueous suspension: Ethacilin® vet (ETH) (Intervet AB, Sweden) and an oily suspension: Ultrapen (UPA) (N-vet, Sweden), both containing 300 mg/mL. Respiratory signs were scored daily using a scale from 0 (absence of disease) to 3 (severe respiratory signs) to assess treatment efficacy. Blood samples were collected to determine plasma concentrations of PC using ultra performance liquid chromatography - tandem mass spectrometer (UHPLC-MS/MS). Pigs were euthanized 16 days after inoculation. This study was approved by the regional ethical committee in Uppsala and by the Swedish Medical Products Agency.

Table 1. Treatments of 30 specific pathogen-free (SPF) pigs inoculated with 10^{11} cfu APP serotype 2 and treated im with different dosings of two formulations of PC (Ethacilin or Ultrapen). Pigs in one group served as an untreated control group (NT)

Group	Product	Dose	Dosing interval
ETH220	Ethacilin	20 mg/kg	12 hours
UPA30	Ultrapen	30 mg/kg	24 hours
ETH30	Ethacilin	30 mg/kg	24 hours
ETH20	Ethacilin	20 mg/kg	24 hours
NT	Untreated	na*	na*

*not applicable

Results

Only Ethacilin 20 mg/kg twice daily and Ultrapen 30 mg/kg once daily resulted in plasma PC exposure adequate for antimicrobial response. This is also demonstrated in clinical scores (Fig. 1). Mean clinical scores are shown by group and day in Figure 1. Clinical scores were lower for all treatment groups compared to untreated pigs ($p< 0.001$). Clinical scores for the ETH220 group were also lower compared to the ETH20 and ETH30 groups ($p< 0.05$).

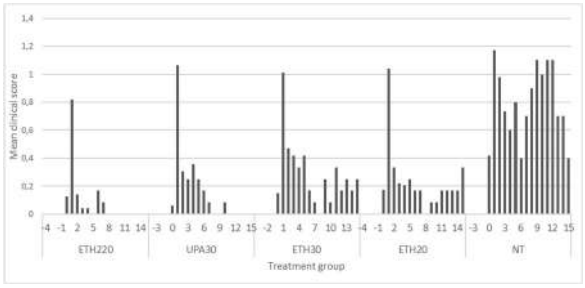

Figure 1. Daily mean clinical scores for 30 SPF pigs inoculated with 10^{11} cfu APP serotype 2 and treated im with different dosings of two formulations of PC (Ethacilin or Ultrapen).). Pigs in one group served as an untreated control group (NT).

Discussion and Conclusions

The results indicate that treatment of APP infections with PC can be improved by more frequent dosing. Thereby, concentrations of PC exceeding MIC of APP for sufficient time of the dosing interval were achieved, which is necessary for time-dependent antibiotics, i.e. β-lactams. The higher dose of the oil-based formulation also appeared to improve treatment efficacy.

Acknowledgments

The Swedish Research Council Formas for funding

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Alternatives to the use of antibiotics in swine production

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Introduction

Antimicrobials have been used for disease treatment, prevention and as growth promoters. Emerging of resistant bacterial and the non-prudent use of antimicrobial are in the core of food safety concern in pork production (4,5). This scenario has motivated research in alternatives that support animal health without jeopardizing performance. Prebiotics, probiotics, organic acids and essential oils may be alternatives to the use of conventional antibiotics (1,2). This study evaluated the substitution of antibiotics routinely used in by feed additives administrated from the nursery to the finishing pig production stages.

Materials and Methods

The experiment started with 1,100 weaned pigs divided into 36 pens with six treatments (6 pens by treatment) during nursery, growing and finishing stages, however, to growing and finishing stages 850 animals remained divided into 36 pens with six treatments (6 pens by treatment). At weaning the animals were divided by sex (females and males) and submitted to the vaccination protocol. Animals were weighed individually to achieve a homogeneous distribution among the 6 treatments, being: **ATBFree:** antibiotic free feed; **ATB**: antibiotic feed; **Prebiotic**: prebiotic feed (mannanoligosacharide MOS); **Probiotic**: probiotic feed (*Bacillus* spp., *B. bifidum, E. faecium, L. acidophilus*); **EO**: essential oil feed (Thymol and Carvacrol) and **OA**: organic acid feed (citric, lactic and ascorbic acids). After the nursery stage, the animals were weighed a second time to determine the daily weight gain (DWG) and conversion rate (CR). During growing and finishing stages the animals were weighed individually another four times.

Furthermore, vaccination protocols were performed for *Mycoplasma hyopneumoniae* at seven days of age, recombinant verotoxin 2e of *E. coli*, *Lawsonia intracellularis*, Porcine Circovirus (PCV2), *Salmonella tiphymurium*, beta-hemolytic *E. coli*, *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis* and *Pasteurella multocida* at weaning. During the experimental period the utilization of parenteral antibiotic treatment was performed as needed for any animal independent of the treatment group. The results were presented as mean ± standard error of the mean. Means were compared through Tukey HSD test. The data were analyzed on Statistix 10.0.

Results

There was no statistical difference between the treatments during the nursery, growing and finishing stages ($p>0.05$, Table 1).

Conclusions and Discussion

It is possible to envision a swine production system without the utilization of prophylactic antibiotics. However, it is necessary to organize the production structure. Among the production practices, the ones that reduce the mixing of different litters when forming pen groups, and stimulate vaccination and sanitary breaks, should be emphasized (3).

Antibiotics are mainly used with a prophylactic emphasis, meaning that healthy animals will also receive the drug. When antibiotic utilization is intense, there is a risk for the microorganisms to select resistant strains, due to the frequent exposure to the drug. The utilization of alternative products has not impacted feed conversion when compared to antibiotics. The companies should adequate to the new production system with the prudent utilization of antibiotics.

Table 1. Feed conversion in pigs subjected to different treatments as substitutes for antibiotics (mean ± standard error of the mean).

Treatment	Feed conversion	
	Nursery (p=0,1969)	Growing and finishing(p=0,8225)
ATBFree	1.58±0.02	2.42±0.04
ATB	1.50±0.04	2.45±0.03
Prebiotic	1.59±0.04	2.39±0.02
Probiotic	1.63±0.05	2.39±0.03
EO	1.61±0.02	2.40±0.05
OA	1.58±0.04	2.44±0.04

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Alternatives to the use of antibiotics in swine production

Marcelo F. Güths[1], Helloa A. Siqueira[2], Julia H. Montes[2], Giovani Nardelli[1], Cleverson Hebbel[1], Maiko G. Philippe[2], Vanessa Peripolli[2], Fabiana Moreira[2], Yuso H. Tutida[1], Elizabeth Schwegler[2], Renato Irgang[3], Jalusa D. Kich[4], Ivan Bianchi[2]

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Antibiotics are mainly used with a prophylactic emphasis, meaning that healthy animals will also receive the drug. When antibiotic utilization is intense, there is a risk for the microorganisms to select resistant strains, due to the frequent exposure to the drug. The utilization of alternative products has not impacted feed conversion when compared to antibiotics. The companies should adequate to the new production system with the prudent utilization of antibiotics.

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Determination of the minimum inhibitory concentration (MIC) in *vitro* of strains of *Lawsonia intracellularis* isolated in Brazil

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Introduction

The treatment of choice for the control of proliferative enteropathy is the use of antimicrobials (1). However, in order to achieve the best possible result, the antimicrobial of choice is essential, so information on antimicrobial sensitivity of different molecules against Brazilian isolates of *Lawsonia intracellularis* still needs to be evaluated. Therefore, the aim of this study was to test new antimicrobials against Brazilian isolates of *L. intracellularis in vitro*, thus increasing the possibility of success in the treatment of the disease.

Materials and Methods

A total of 5 antimicrobials (Tiamulin, lincomycin, ciprofloxacin, spectinomycin and gamithromycin) were tested in this study using two Brazilian *L. intracellularis* strains (PHEBR_E5 and PHEBR_E8). The stock solutions were prepared in a final concentration of 2560 µg/mL, and 12,800 µg/mL. For the tests, the antimicrobials were diluted in culture medium in the final concentrations of 0.125; 0.25; 0.5; 1; 2; 4; 8; 16; 32; 64 and 128 µg/mL and tested in triplicate. To determine the minimal inhibitory inhibition (MIC) of *L. intracellularis in vitro*, the intracellular and extracellular activities were evaluated, using previously described assays (1).

Results

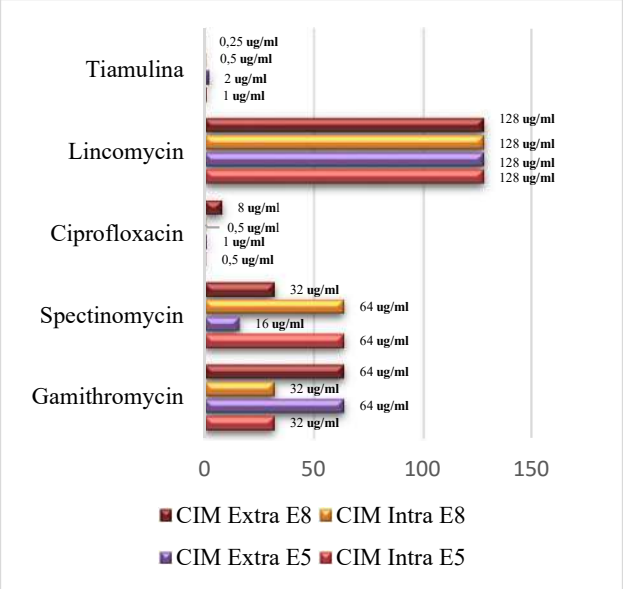
The results obtained for extracellular and intracellular MIC of all antimicrobials are listed in Figure 1. Ciprofloxacin and tiamulin showed the best results for both *L. intracellularis* strains, exhibiting the highest activities for both intracellular and extracellular tests, gamithromycin and spectinomycin showed moderate activity, for PHEBR_E8 and PHEBR_E5 strains, with some differences in concentration for both intracellular and extracellular assays. Finally, lincomycin showed the lowest activity for both strains in intracellular and extracellular tests.

Conclusions and Discussion

Only four *in vitro* sensitivity studies of *L. intracellularis* strains have been published. One used only European isolates (1), other used European and North American isolates (2), a third used Asian isolates (3) and the last used Thai and Brazilian isolates (4). The results of the present study corroborate the findings of the North American isolates tested previously related to tiamulin, lincomycin and spectinomycin (2,3). This is the first time results for in vitro sensitivity to ciprofloxacin, and gamithromycin are demonstrated.

In conclusion, the *in vitro* data detected are promising and bring new options for the treatment of proliferative enteropathy in Brazil; however, it becomes important and requires the continuation of studies, mainly *in vivo* tests to determine the interaction of molecules or that determines the final potential of the effect of these antimicrobials.

Figure 1. Antimicrobials with the different (MIC), intra and extracellular, for the two Brazilian strains of *L. intracellularis*



Acknowledgments

This study was supported by Agroceres Boehringer Ingelheim Saúde Animal, Ceva Saúde Animal – Brasil. Agroceres Multimix Nutrição Animal Ltda.

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Minimum Inhibitory Concentrations of Tylvalosin against Recent Field Strains of *Mycoplasma hyopneumoniae* Isolated from Four European Countries

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Introduction

Mycoplasma hyopneumoniae is a primary pathogen within the Porcine Respiratory Disease Complex (PRDC) which leads to health and welfare problems and poor performance. Even with multifaceted approaches to the prevention and control of PRDC on farm, including biosecurity and vaccination, treatment with antibiotics is often necessary. Although macrolides are generally highly effective against *M. hyopneumoniae*, sensitivity testing is helpful in selecting antibiotics and monitoring their efficacy. The objective of this study was to determine the Minimum Inhibitory Concentrations (MICs) of the macrolide tylvalosin (TVN), the active ingredient in Aivlosin® (ECO Animal Health Ltd), against recently isolated European strains of *M. hyopneumoniae*.

Materials and Methods

Test article (TVN) was tested for purity, dissolved in sterile deionized water and stored frozen prior to use. Multiple strains of *M. hyopneumoniae* were obtained from collections of recently isolated field strains from pigs with signs of respiratory disease: 10 from Spain (isolated from 2010-2011), 10 from Hungary (isolated 2015), 10 from Belgium (isolated 2015), and 10 from the UK (isolated 2016). In addition, the reference strain NCTC 10110 was used to monitor the performance of the tests. Prior to use, all strains were stored in cryoprotective suspension at -80°C. The test system used broth microdilution MIC methodology[1,2] modified for suitability for *M. hyopneumoniae*[3]. Culture media were prepared using supplemented Friis medium adjusted to pH 7.6. Inoculated plates were incubated at 37°C. Sterile microtitre plates were prepared with serial doubling dilutions of test article in 100 µL aliquots of Friis medium to give a concentration range from 0.001 to 4 µg/mL. Each plate included wells acting as positive or negative controls. Wells were inoculated with 100 µL of broth containing approximately $1x10^6$ cfu per mL of each strain of *M. hyopneumoniae*. Plates were read at 24 h intervals and incubated for 4-18 days until all strains produced MIC results. The MIC range, MIC_{50}, and MIC_{90} were calculated for each test article.

Results and Discussion

The results are presented in Table 1. The range of MICs for TVN against all strains of *M. hyopneumoniae* tested (including the reference strain) was 0.008–0.031; these were within ±1 doubling dilution of the median which demonstrated the consistency of the test system. These results are comparable to those reported previously for TVN for isolates collected between 1997 and 2000 (MIC_{90} of 0.06 µg/mL)[4] and for those reported in 2014 (MIC_{90} of 0.06 µg/mL)[5], indicating that to date there has been no relevant change in susceptibility over time.

Table 1. MIC results for 40 European strains (10 per country) of *M. hyopneumoniae*.

	TVN MIC (µg/mL)		
	Range	**MIC_{50}**	**MIC_{90}**
Spain	0.016 – 0.031	0.016	0.031
Hungary	0.016 – 0.031	0.016	0.031
UK	0.008 - 0.031	0.016	0.031
Belgium	0.016 – 0.031	0.016	0.031
All	0.008 - 0.031	0.016	0.031

Conclusions

The susceptibility to TVN of these recently collected *M. hyopneumoniae* field strains from four European countries was consistently high. These data will be useful in the ongoing monitoring of Aivlosin® effectiveness for the management of PRDC in the field. The susceptibility to TVN established in this survey indicates no change over time when compared to previously generated MIC data.

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A standardized broth microdilution technique to evaluate antimicrobial susceptibility of *Brachyspira hyodysenteriae* against essential oils

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Introduction

The rising of bacterial antimicrobial resistance has led to an increasing concern about the use of antimicrobials in animal production. Therefore, the activity of nutraceuticals such as essential oils (EO) has been evaluated recently[1,2]. The causative agent of swine dysentery (SD), usually controlled with in feed antimicrobial, is an anaerobe Gram-negative spirochete named *Brachyspira hyodysenteriae*[2]. The objective of the present study was to evaluate the antimicrobial activity of thymol, carvacrol and cinnamaldehyde against *B. hyodysenteriae* in a broth microdilution technique and to compare this technique with the commercial kit (VetMIC™ Brachy SVA)

Materials and Methods

Nine Brazilian strains of *B. hyodysenteriae*, previously isolated in our laboratory, and a reference strain were used. The strains were plated on Tryptic Soy Agar complemented with 5% ovine blood, maintained under anaerobiosis atmosphere at 37°C for four days. Then, resuspended in Brain Heart Infusion Broth complemented with 10% of fetal bovine serum, using the 0,5 McFarland scale to standardize the suspension. Finally, the strains were propagated under anaerobiosis atmosphere at 37°C for four days in 96 wells plates, with serial dilutions of the following EO: thymol, carvacrol and cinnamaldehyde. Antimicrobial sensitivity was evaluated by bacterial growth, visible through the media turbidity. The same *B. hyodysenteriae* strains were evaluated using a commercial kit VetMIC™ Brachy SVA (National Veterinary Institute, Uppsala, Sweden) and in a broth microdilution with serial dilution of lincomycin, doxycycline, tiamulin, valnemulin and tylosin.

Results

The minimum inhibitory concentrations (MIC) 50 and 90 are shown in table 1 for all the antimicrobials. MIC values obtained in microdilution method were within the range of one dilution below or above, proposed elsewhere[4,5], when compared to the former commercial kit (VetMIC™ Brachy SVA).

Conclusions and Discussion

This study was the first to standardize a broth microdilution test to evaluate the antimicrobial sensitivity of *B. hyodysenteriae* in Brazil and the fourth worldwide to evaluate the antimicrobial activity of essential oils against *B. hyodysenteriae*.

The broth microdilution test allows a wide range of dilutions, at the researcher's choice, that it is not possible with the former commercial kit, since the antimicrobials and dilutions are pre-determined, with eight dilutions available. The microdilution test allowed 11 dilutions (in this study, ranging from 256µg/ml to 0,25µg/ml). In addition, only six antimicrobials can be tested with the commercial kit, while using the microdilution method, any soluble compound can be evaluated.

Table 1. MIC 50 and MIC 90 values for the microdilution (MD) and commercial kit, expressed in µg/ml

Antimicrobial	MD[1]		VetMIC™	
	MIC 50	MIC 90	MIC 50	MIC 90
Lincomycin	16	32	32	64
Doxycycline	1	1	2	2
Tiamulin	16	32	>8	>8
Valnemulin	4	8	4	>4
Tylosin	>256	>256	>128	>128
Thymol	128	256	NA	NA
Carvacrol	128	256	NA	NA
Cinnamaldehyde	128	128	NA	NA

[1] MD – Microdilution, NA – Not applicable

The MIC values for the EOs were within others studies[6]. These results may not represent the actual antimicrobial activity of EOs, since other mechanisms has been proposed (anti-inflammatory, microbiome modulation, gut function[2]) and further studies *in vivo* are necessary to evaluate the antimicrobial activity of essential oils.

Acknowledgments

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Analysis of antibiotic use and cost in *Mycoplasma hyopneumoniae* negative and positive pig flows

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Introduction

Antibiotic resistance is reason for major concern both in humans and animals. Public health experts are working with physicians and veterinarians to minimize inappropriate antibiotic usage in their patients (1,2,4). Understanding the current pattern of antibiotic use in livestock is important to support antibiotic use, which may potentially slow down the emergence of multi-antibiotic resistant microorganisms in animals (4). *Mycoplasma hyopneumoniae* (M. hyo) has been demonstrated to be an important infectious agent that can result in increased antibiotic use (2). The goal of the present study was to compare the use of antibiotics in M. hyo negative and positive pig flows in Brazil, assessing the kg of medicated feed, mg per kg of live weight, and antibiotic cost.

Materials and Methods

During the second half of 2019, information on the routine use of therapeutic antibiotics in feed was analyzed in 23 pig flows produced by 336,500 sows from different Brazilian companies, located in seven (7) states: Parana (41%), Rio Grande do Sul (32%), Santa Catarina (15%), Mato Grosso do Sul (6%), São Paulo (2%), Mato Grosso (2%), and Minas Gerais (1%). Out of this total, 5 monitored pig flows are M. hyo negative and represent 13,600 sows. It is important to highlight that the Brazilian herd is negative for the PRRS virus. The analysis considered the use of therapeutic antibiotics in the feed during growth, from weaning to a final live weight of 122.0 kg. The economic analysis considered a common price for the antibiotics used in all herds.

Results

Results showed that pigs from M. hyo negative flows received in average 34% less medicated feed, and 48% less antibiotics measured in mg per kg of live weight when compared to the positive flows. In the general analyses, the range of antibiotic use varied from 23 kg to 239 kg of medicated feed, and from 117 mg to 514 mg per kg of live weight (table 1). However, a huge difference was seen in the expenditures to cover the cost of antibiotics, up to 12 times. Again, an economic advantage was observed for M. hyo negative pig flows, as the investment was 2.6 times lower, when compared to positive flows.

Table 1. Antibiotic use in M. hyo positive and negative pigs flows in Brazil

	Medicated Feed (kg/Pig)		ATB (mg kg LW)		Cost (Times)
Pig´s Flows	Average	Range	Average	Range	Gap
M hyo (+)	113,3	65 - 239	322	125 - 514	1 - 5,0 x
M hyo (-)	74,8	23 - 124	167	117 - 226	1 - 4,0 x
Dif.	34,0%	23 - 239	48%	117 - 514	1 - 2,6 x

Conclusions and Discussion

This was the most encompassing study on the use of antibiotics conducted in Brazil, covering close to 18% of the total Brazilian herd and the main producing states. The low number of sows in the M. hyo negative flows represents a Brazilian reality. In this study, the measured result of 316 mg of antibiotic per kg of live weight was 11.7% lower than in a previous study in Brazil (2). Bondt et al 2013 highlighted the importance of establishing a methodology for comparing the use of antibiotics between countries in order to avoid misinterpretation (1). A simple comparison between countries, based only on total antibiotics sales, entails the risk of serious misinterpretations (1). A lower antibiotic usage and cost was seen in M. hyo negative herds, proving this to be one of the most relevant strategies. Biosecurity measures to maintain high health status in the herds are fully justified, not just in view of the worldwide concern about antibiotic use (3, 4), but as well as an opportunity to reduce production costs, as it was seen in this current study. The combined assessment of medicated feed usage (kg feed / pig), mg per kg live weight and investment costs ($ / pig) can be one of the strategies used for comparing different production systems. In order to reduce the use of antibiotics, M hyo negative herds should be prioritized, especially when considering new projects.

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Antimicrobial susceptibility profile of Brazilian *Mycoplasma hyopneumoniae* isolates

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Introduction

Mycoplasma hyopneumoniae is the etiological agent of enzootic pneumonia (EP), a worldwide chronic respiratory disease of swine. Infected pigs are prone to secondary infection, reduced weight gain and eventually require antimicrobial treatment aiming to reduce the impact on production and profitability (1) . Vaccination is at the core of strategies to control infections and to reduce pathological lesions observed during outbreak. In addition, pulse medication with antimicrobials is still used to control *M. hyopneumoniae* infection in growing and finishing pigs triggering the possible emergence of resistant strains. In this sense, here we evaluated the susceptibility profile of twenty-one Brazilian clinical isolates of *M. hyopneumoniae* to 11 antimicrobial drugs commonly used in pigs.

Materials and Methods

Twenty-one strains of *M. hyopneumoniae* isolated from clinical cases belonging to AFK Imunotech (*n*=15) and CEDISA (*n*=6) collections were included in this study. The bacteria were isolated from lungs with gross lesions suggestive of EP obtained in slaughterhouse (pig with 150 to 180 days old) from January to November of 2019. The isolates are geographically located in South Brazil (Fig.1), the most important region of pig production.

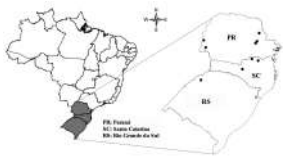

Figure 1. Geographical distribution of *M. hyopneumoniae* isolates (dots).

The identity and purity of all isolates were confirmed by real time polymerase chain reaction (2). The antimicrobial susceptibility test was carried out using the microbroth dilution method to determine the minimum inhibitory concentration (MIC) as previously described (3). Solutions of 11 antimicrobials (pure powder from Sigma-Aldrich, USA) were prepared as recommended by CLSI (CLSI, 2013) to give a final active concentration range from 0.03 to 64 µg/mL. To each plate three different controls were included: positive growth (Friis medium + *M. hyopneumoniae* - isolates), Friis control (Friis non-inoculated), antimicrobial control (Friis medium + antimicrobials). *Staphylococcus aureus* ATCC 29213 was used to validate the antimicrobial concentration. All isolates were tested in duplicate and the MIC of each antimicrobial was recorded as the lowest concentration that completely inhibited growth.

Results

The MIC distribution is indicated in table 1. *M. hyopneumoniae* isolates had high susceptibility to Tiamulin, Ciprofloxacin and Enrofloxacin. Intermediate susceptibility was found for Florfenicol, Lincomycin, Marbofloxacin and Oxytetracycline and a decreased susceptibility pattern was observed for Erythromycin, Tilmicosin, Tylosin and Tulathromycin.

Table 1. MIC values for 11 antimicrobial agents against 21 *Mycoplasma hyopneumoniae* isolates.

Antimicrobials	MIC parameter (µg/mL)		
	MIC Range	MIC_{50}	MIC_{90}
Tiamulin	≤0.03 – 1	0.12	0.25
Ciprofloxacin	≤0.03 – 1	0.25	0.5
Enrofloxacin	≤0.03 – 2	0.5	1
Florfenicol	0.03 – 64	2	4
Lincomycin	0.06 – 8	0.5	2
Marbofloxacin	0.03 – 4	1	2
Oxytetracycline	0.03 – 4	1	4
Tilmicosin	0.06 – >64	1	32
Erythromycin	2 – >64	32	64
Tylosin	≤0.03 –>64	0.12	64
Tulathromycin	≤0.03 –>64	0.03	64

Conclusions and Discussion

Our results indicate that the use of three macrolides to treat *M. hyopneumoniae* infection should be of major concern. Tylosin was recently described to be effective only in high concentration against Brazilian *M. hyopneumoniae* isolates (4), strengthening our results. Because of the wide susceptibility variation amongst antimicrobials, the constant use of MIC to monitoring the isolates is pivotal to avoid the surge of resistant strains in the field.

Acknowledgement

This work was supported by AFK and CEDISA.

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Antimicrobial resistance and biofilm production by *Streptococcus suis* isolated from pigletes

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Introduction

Streptococcus suis is considered a re-emerging zoonotic pathogen that can cause severe diseases (3): septicemia, pneumonia, endocarditis and arthritis but mainly meningitis in pigs and humans, depending on the *Streptococcus* serotype (5). Biofilms are perfectly structured microbial communities that are found in a matrix and that adhere to biological surfaces. The matrix they produce protects microorganisms against host defense mechanisms, antibiotic treatment. Bonifait *et al* (2008) was the first to report biofilm production by *S. suis*. In recent years, antibiotic resistance levels have increased worldwide, representing a public health problem. One of the main problems in the treatments used for *S. suis* is the formation of biofilm and the existence of persistent microorganisms (4). The objective of the work was to know the antimicrobial resistance and biofilm production of *S. suis* strains isolated from diseased piglets.

Materials and Methods

The material consisted of sixteen samples collected from piglets. Biofilm formation was assessed in 96-well pollystyrene tissue culture microplates. The *Streptococcus* isolates were inoculated into BHI and incubated at 37 °C. After incubation content of each well was gently removed by tapping the plates. The wells were washed three times with PBS pH 7.2 to remove free floating planktonic bacteria. Biofilm were stained with crystal violet 0.1%. Optical density of stained adherent bacteria was determined with a micro ELISA reader a 492 nm. Experiment was performed in duplicate and repeated three times. The Kirby Bauer technique was used to detect resistance to Tetracycline, Ampicillin, Cephalothin. Cefotoxime, Dicloxacillin, Ciprofloxacin, Clindamycin, Erythromycin, Gentamicin, Oxacillin, Ceftiofur, the zones of inhibition were measured to be classified as sensitive, intermediate and resistant according to the criteria of the Clinical and Laboratory Standars Institute.

Results

In the DO 550 absorbances obtained in the plaque test, 4 strains (25%) were found with aborbances of 0.3, 4 (25%) strains with absorbance greater than 0.2 and the rest of the 50% strains (8/16) with absorbances greater than 0.1. 100% resistance was found to all antimicrobials tested in the strains.

Conclusions and Discussion

The strains of *Streptococcus suis* evaluated were shown to have biofilm formation capacity, which evidences the virulence of the strains. Dawei *et a*l. found 10.7% of *Streptococcus suis* strains that had an OD greater than 0.1 and 89.3% of the strains had an OD less than 0.1, unlike what was found in this study in which 50% had absorbances greater than 0.2. Biofilm formation in *S. sui*s has recently been reported in isolated field strains suggesting its importance in pathogenesis, early detection and proper management of potentially pathogenic *S. suis* strains may be a first step in prevention and management.of infections associated with this microorganism. It is important to take measures to control the use of antimicrobials in pig units because very high rates of resistance were observed than reported in other countries.

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Risk factors for antibiotic treatment in two Danish herds raising pigs without antibiotics

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Introduction

In Denmark, raised without antibiotics (RWA) is a new production concept where all pigs are ear-tagged at birth with a RWA ear-tag (1, 2). If pigs require any antibiotic (AB) treatment during rearing, the ear-tag is removed and they lose their RWA status. High focus on hygiene, management, vaccines and feeding, characterize herds with RWA production (1). The objective of the study was to identify when pigs receive their first AB treatment and lose their ear-tag, and identify risk factors that characterized pigs receiving treatment.

Materials and Methods

The study was performed in two Danish RWA herds with Danbred pigs from birth to around 12 weeks of age. Newborn piglets received an ear-tag with individual ID, in the opposite ear of their RWA ear-tag. Bodyweight (BW) was recorded at birth, two weeks, four weeks and 12 weeks of age. Risk factors from both sow and piglets were recorded every week from birth to 12 weeks of age. The risk factors included, parity, litter size, live born, dead born, birth weight, gender, weaning age, weaning weight, BW, moves between sows and moves between pens.

The effect of AB treatment on BW was analysed in a mixed linear model in R. Categorical risk factors for AB treatment were analysed in a logistic binomial model in R.

Results and Discussion

The study included 518 piglets from herd A with a mean birthweight of 1.24 kg and 436 piglets from herd B with a mean birthweight of 1.27 kg. Herd A ear tagged all piglets at birth while herd B did not ear tag the smallest 54 piglets. Regardless of strategy, 64-68% of the piglets born remained untreated until 12 weeks of age (Fig 1).

In herd A the untreated pigs had a significantly (P < 0.001) higher BW at two and four weeks of age, whereas there was only a tendency (P = 0.071) towards a higher BW at four weeks of age in herd B. At 12 weeks of age no significant difference in BW was observed in either herd. In herd A, 11% died and 6.7% were missing at 12 weeks of age, whereas 8.5% died and 5.3% were missing in herd B.

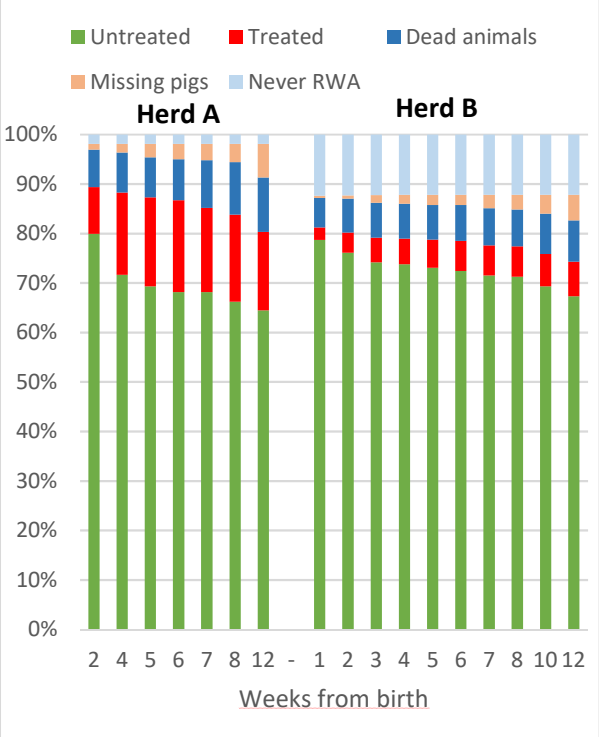

Figure 1. The percentage of untreated, treated, dead, missing and never ear-tagged for RWA pigs in the two herds.

In herd A, pigs with a BW below 4.5 kg at weaning, had a higher risk of receiving AB treatment before both weaning (Odds ratio 32.8, P < 0.001) and 12 weeks of age (Odds ratio 33.3, P = 0.020). Whereas in herd B, male pigs had an increased risk of receiving AB treatment before weaning (Odds ratio 4.1, P = 0.019).

Conclusions

Approximately 64-68% of Danish pigs in two RWA herds were untreated with AB at 12 weeks of age and therefore still RWA-pigs. The smallest pigs at weaning as well as male pigs were at a higher risk of AB treatment.

Acknowledgements

Student and staff at the University of Copenhagen, and the staff at the two study herds.

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How to measure the link between farmers' trust and their compliance to veterinary advice in the French pig sector?

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Introduction

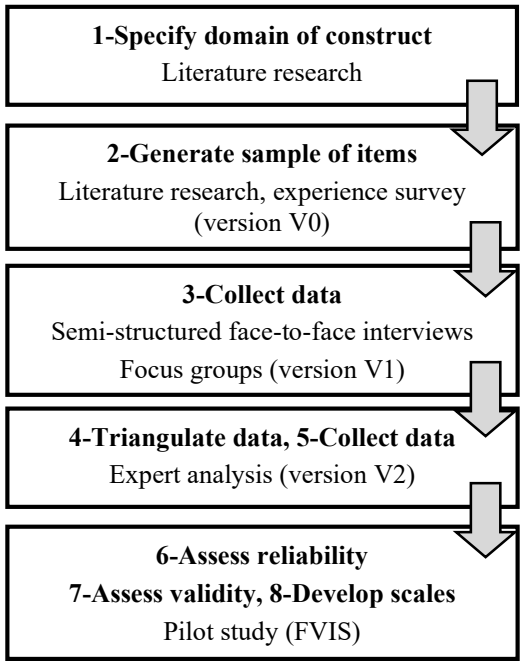
The co-design and the co-development of innovative healthcare strategies are essential for a cautious use of antimicrobials in pig farming. To implement new strategies that are adapted to local contexts, interaction and interpersonal trust between farmers and veterinarians are key.

In human healthcare, numerous studies argue that patient's trust in their physician is considered essential for good quality and effective medical care (1). Drawing on the work done in the human health sector (2 3), we developed a Farmer-Veterinarian Interaction Scale (FVIS) to assess the link between farmers' trust in veterinarians and farmers' compliance to veterinary advice in the French pig sector. This, we believe, could foster comprehensive dialogue between farmers and veterinarians for more sustainable pig health management and antimicrobial use in farming.

Materials and Methods

The construction of the scale relied on the Churchill method (Figure 1) (4).

Figure 1 – Churchill's method (4)



A first version (V0) of FVIS was established based on a review of the literature in the field of clinical trust and patient-doctor interaction scales. Then, we conducted four focus groups with pig veterinarians (n=16), as well as 21 semi-structured face-to-face

interviews with pig farmers (n=26) to explore farmers' and veterinarians' views on trust and revise V0. After triangulating our data with 25 pig experts, we obtained V2. Finally, we tested V2 in the field with five pairs of farmer-veterinarian to obtain a final version of the scale.

The different qualitative steps had several aims. First, to adapt the scale to the specific context of the French pig sector. Second, to avoid any redundancies between items. Third, to clearly define each item used in the scale with the help of different actors from the French pig industry (3).

Results

In total, 41 items were retrieved from the literature for V0 and categorised into seven dimensions of interpersonal trust, namely 'Competence', 'Integrity', 'Honesty', 'Confidentiality', 'Fidelity', 'Caring', and 'Global trust'.

Eleven more items and one additional dimension of trust ('Availability') were added to V1 as a result of the focus groups and the qualitative interviews. This concept emerged as essential for farmers and is now described in the scale by four items. The dimension 'Competence' was also completed with four items to emphasise the importance of veterinarian's communicational skills. The dimensions 'Integrity' and 'Honesty' were both merged because their distinction was unclear to farmers and veterinarians. After qualitative evaluation by 25 pig experts, 42 items were selected to be included in V2. The pilot study allowed us to test and validate a final version of the scale with 30 items.

Conclusion

FVIS is the first scale validated in the pig sector in France to explore farmer-veterinarian trust in animal health management. It will be used in a future cohort study with 30 pairs of farmer-veterinarian to explore the relationship between trust and pig farmers' compliance to veterinary advice.

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Pig farmer's perceptions, attitudes and management toward antimicrobial usage

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Introduction

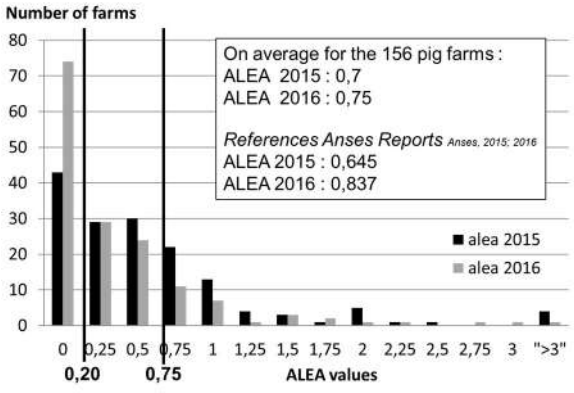
Because of the rising threat from antimicrobial resistance, a number of international, European and national initiatives have been developed to mitigate the risk from antimicrobial use in animals. However, these initiatives are facing challenges and information gaps, especially related to how to quantify, explain and reduce antimicrobial use in food-producing animals. On field, pig farmers are strongly encouraged to reduce their antimicrobial usage. In order to achieve national and European reduction targets, herd level action is needed. Alternative, especially preventive measures have to be implemented to reduce the need for antimicrobial treatments. Nevertheless, what is the reality?

In order to update data concerning the different practices and perception for antimicrobial usage in pig farms, this study aimed at comparing antimicrobial usage, technical performances, management practices, farmers' perception of their antimicrobial usage and farmers' attitudes toward antimicrobial resistance

Materials and Methods

The survey was carried out in 20 selected pig farms located in the West region in France among 156 monitored since 2015 with Indicavet. This software monitored the antimicrobial usage of pig farms and is based on vet prescriptions The Animal Level of Exposure to Antimicrobials (**ALEA**) is calculated by dividing the body weight treated by the biomass of the animal population potentially treated with antimicrobials. Four antimicrobial consumption categories based on two threshold ALEA values (0,2/0,75) : low consumers, high consumers, farms who decrease their antimicrobial consumption between 2015 and 2016 and farms who increase their antimicrobial consumption (Figure 1).

Figure 1. ALEA values' distribution for the 156 eligible farms monitored since 2015 with Indicavet



After the selection of five farms per category, a questionnaire has been filled during an interview with the farmers The association between practices, perceptions or technical performances (feed conversion ratio, wean-to-finish mortality rate) has been analyzed with Chi Square or Kruskal-Wallis tests

Results

No link has been seen between technical performances and antimicrobial usage. Vaccination is considered as a major tool of antimicrobial reduction like biosecurity (17/20) or the use of "alternatives" (15/20) (Table 1). Nevertheless, during an opened question concerning the ineffective measures, these «alternatives» have been cited (5/20). In all the interviews, pig farmers underline the strong advisory role of the veterinarian especially for strategic decision-making resulting in antimicrobials usage and state that antimicrobial resistance is a main concern. Among the farmers' proposals as key measures for antimicrobial reduction, a better training and more informations/knowledge concerning diseases, treatments or alternatives were cited (8/25).

Table 1. Major tools for effective antimicrobial reduction according to pig farmers

Major "tools" for antimicrobial reduction	Answers of the farmers	
	NO	YES
Vaccines	3	17
Biosecurity	3	17
Use of alternatives	4	15
Sanitary status	7	13
Regulation/Prices	8	12
Building/Material	11	9
Genetic	13	5

Conclusions and Discussion

This study highlight brakes and levers for antimicrobial reduction in pig farms. Many positive and encouraging points like the strong implication of the veterinarian as the main advisor on animal health.

Strenghtened advisory role implies that veterinarians develop better communication skills and adjust their advices to the perceptions or attitudes of the farmers.

Finally, the absence of link between the technical performances and the consumption of antimicrobials is also a major point to help veterinarians to engage farmers to reduce their antimicrobial use. This is a key to help farmers to comply with the measures they recommend whatever the economic situation.

Antimicrobial usage evolution between 2010, 2013 and 2016 in a group of French pig farms

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Introduction

In France, the Ecoantibio plan is a success : pig exposure to antimicrobials declined by 41 % during the Plan's five years (2012-2016), which is far beyond the initial -25% objective. But a question arises : is this evolution similar in all pig farms ?

The objective of this study was to analyse the individual trajectory of each farm concerning antimicrobial usage between 2010, 2013 and 2016 and to identify the factors of variations.

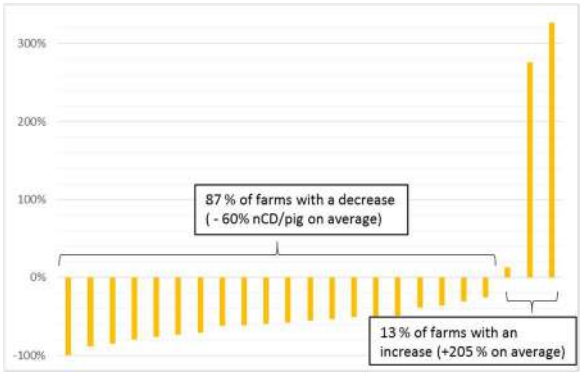
Materials and Methods

The study monitored antimicrobial usage by weight group in 33 farrow-to-finish farms in western France between 2013 and 2016. Among them, 23 farms were also included in a precedent study that monitored their antimicrobial usage in 2010 (Hémonic et al., P127, ESPHM 2015). Antimicrobial usage was quantified by the number of Course Doses per produced pig per year (nCD / pig). At a farm level, an evolution ≥ ± 1 nCD/pig was considered as a significant decrease or increase. Otherwise, the evolution was considered as stable. At a weight-group level, the threshold was fixed at ± 0.5 nCD/pig.

Results

In the 23 farms monitored between 2010-2016, the decrease of antimicrobial usage over seven years was marked (- 60% on average) and concerned a large majority of farms (87 %) (Figure 1).

Figure 1. Variation (%) of nCD/pig between 2010-2016 in 23 farms

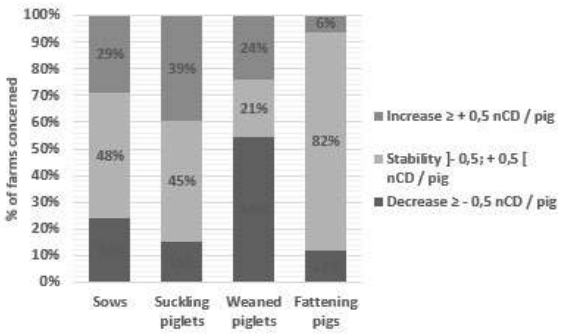


After classifying the farms according to the evolution of their antimicrobial usage during the first period (2010-2013) and the second period (2013-2016), only eight farms (35 %) kept the same type of evolution over both period : decrease, stagnation or decrease (Table 1). Among the 10 farms with a stable use over 2013-2016 after a reduction use over 2010-2013, four were in the bottom-third group of users in 2013.

So, their margin of reduction was very thin. For the six farms in the middle-third or top-third group, a theoretical decrease was possible but not reached, probably because of their sanitary situation.

On the 33 farms followed between 2013-2016, 12 reduced their AMU : six belongeg to the top-third group of users in 2013 and only two to the bottom-third group. At a weight-group level, only antimicrobial usage for weaned piglets was more frequently reduced between 2013 and 2016 (54 % of the farms). For sows, suckling piglets and fattening pigs, most of the farms had stable usage between 2013-2016 (Figure 2).

Figure 2. Classification of the 33 farms according to their evolution over both periods (2013-2016)



Conclusions and Discussion

All these results highlighed the variability of individual trajectories in antimicrobial usage. This is due to sanitary issues that may be different according to each farm and each period. This is also explained by the level of antimicrobial usage reached at the end of a period : the lower it is, the more difficult it is to continue to decrease during the subsequent period.

This study highlights the inter- and intra-farm variability in the evolution of antibiotic use. So, it is important to monitor the use of antibiotics at the farm level to follow their individual trajectories and to compare them to collective trajectories. The new GVET approach, for example, allows French farmers to self-assess their use of antibiotics and, more generally, of medicines (vaccines, dewormers ...). This usefully complements the monitoring of average evolution at the country level.

Acknowledgments

Ecoantibio Plan 2017.

Comparison of standardized methods for antimicrobial susceptibility testing of *Streptococcus suis* field isolates to amoxicillin

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Introduction

Antibiotic treatment is still important tool in order to control many bacterial infections in pigs, for which commercial effective vaccines are not available. The disease caused by virulent serotypes of *Streptococcus suis* is one of the most important septicaemic infection of piglets before weaning (10-14 days) and during the nursery period. The performance of antimicrobial susceptibility testing (AST) is important for confirmation of susceptibility to chosen antimicrobial agents and for detection of resistance or types of resistance (1). The AST has to be performed in accordance with internationally accepted procedures.

The aim of the present study was comparison of results of AST of clinical isolates *S. suis* from Czech Republic to amoxicillin using agar dilution method and broth microdilution method with results obtained using by commercially available E-test.

Materials and Methods

Fifteen field isolates of *S. suis* were selected from clinical samples of diseased pigs in period of 2018-2019 in farms in Czech Republic. The individual isolates of *S. suis* representing different farms. The identification of genus and species were performed by mass spectrometry (MALDI TOF) and all isolates were serotyped by coagglutination test or PCR. Agar dilution test and microdilution test were performed strictly according to procedures described (2,3). E-test was performed according to recommendations of manufacturer.

Comparisons between methods were performed by using the Bland-Atman method (4). GraphPad Prism (version 8, GraphPad software inc.) and Sigmaplot (version 14, Systat software inc.) were used.

Results

No resistant isolates of *S.suis* were detected by any of selected methods.

For both dilution methods, MIC_{90} = 0.03 µg /ml for amoxicillin was obtained and remains in the sensitive range according to clinical interpretive criteria established for amoxicillin, as well below the proposed epidemiological cut- off value (ECOFF) 0.5 µg/ml. MIC_{90} 0.047 µg/ml obtained with the E-test was very similar to one obtained by dilution methodology (Figure 1,2).

The mean and median MIC values for amoxicillin obtained with E-test were slightly but non-significantly higher than the mean and median values
obtained with microdilution. Generally, higher values were obtained with Agar dilution (Figure 2). All values were within the limits of confidence intervals and the mean bias was low, 0.0013 and -0.0050, respectively for E-test versus microdilution and E-test versus Agar dilution.

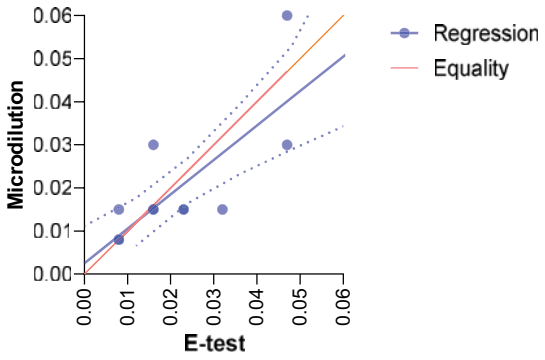

Figure 1: Correlation between MIC values obtained with E-test and Micro-dilution methods (Regression).

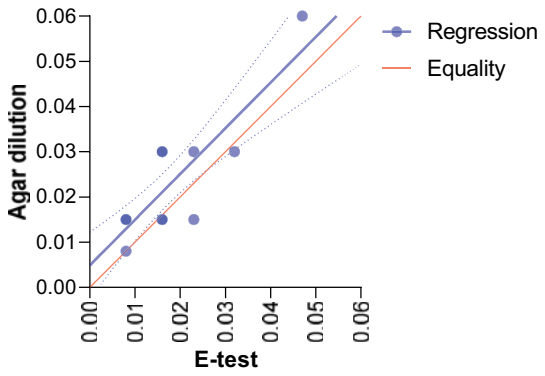

Figure 2: Correlation between MIC values obtained with E-test and Agar dilution (Regression).

Conclusions and Discussion

We have found in our study good agreements between the evaluated methods. E-test could be easy to perform alternative to gold standard dilution methods, with reliable results for sensitivity testing of *S. suis* isolates to amoxicillin.

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Antimicrobial resistance of *Glaesserella (Haemophilus) Parasuis* field strains in Spain

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Introduction

Glaesserella (Haemophilus) Parasuis is a main pathogen affecting pig industry, causing meningitis, polyserositis, polyarthritis and bacterial pneumonia; an infection known as Glässer's disease (1). Antimicrobials have been commonly used to treat this bacterial disease in farm animals, but the emergence of antimicrobial resistance, a serious threat for public health, presses for the implementation of alternatives for disease control (2).

The World organization for animal health (OIE) standards provide global recommendations for controlling antimicrobial resistance, including lists of antimicrobial agents of veterinary importance to treat animal diseases. In parallel, The World Health Organization (WHO) has also developed a list of critically important antimicrobial agents in human medicine. The overlap of critical lists for human and veterinary medicine leads to guidelines, such as the European one (2015/C 299/04), for more restricted antimicrobial interventions for a sustainable pig production. The objective of the present study was to assess the antimicrobial resistance of field strains in Spain and assess what category are they in.

Materials and Methods

A total of 57 samples of *G. Parasuis* obtained from Spanish farms were analyzed. Antimicrobials tested were: florfenicol 30µg (FFC), sulfametoxazole-trimetroprim 25µg(SXT), doxycycline 30µg (DO), tetracycline 30µg (TE), enrofloxacin 5µg (ENF), amoxicillin 25µg (AML), flumequine 30µg (UB), gentamicine 10µg (CN), neomicine 10µg (N), colistin 10µg (CT), ceftiofur 30µg (CEFT).

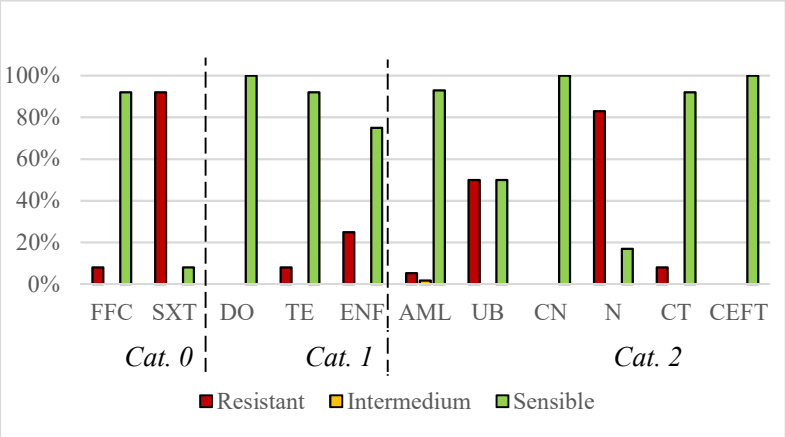

Figure 1. Antimicrobial resistance by categories

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Antimicrobial categories based on Spanish national AMR surveillance plan (PRAN) were: Cat. 0 (Antimicrobials that have not been valued since there are alternatives available to treat serious human diseases, and have less irrigation in the appearance and dissemination of resistances), Cat. 1 (Antibiotics used in veterinary medicine on a regular basis and as 1st choice but they have recommendations of use since they are antibiotics critically important for the Human health), Cat. 2 (Antibiotics that should be used in veterinary medicine as 2nd choice and / or last resort for being antimicrobial critically important for human health).

Results

Field data indicate that antimicrobial treatment can select resistant strains of *G. parasuis*, and can lead to drug and multidrug resistance in *G. parasuis* isolates (Figure 1). It is particularly concerning that two out of three antibiotics for which high resistance were reported in ≥ 50% of the strains (Flumequine and Neomicine) are classified on the PRAN as category 2 (2nd choice or last resort use in veterinary medicine) and categorized by the OMS as Critically Important of priority 1 and 2.

Conclusions

Strategic antimicrobial treatments using antimicrobials may only be advised on a few limited situations, mainly to treat piglets in during a disease outbreak, which is important not only for health but also for welfare issues. Alternative control measures should be taken to minimize the potential increase of Glässer's disease caused by resistant *G. parasuis*.

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Authors would like to thank HIPRA DIAGNOS service for providing information on antimicrobial resistances.

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Multidrug-resistant *Salmonella* sp. isolated from pig slaughtered in Minas Gerais, Brazil

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Introduction

Between 2009 and 2018, 6,809 outbreaks of foodborne diseases were reported in Brazil, of which 11.3% were caused by *Salmonella* sp. and 2% were associated with the consumption of pork and meat products (1). The indiscriminate use of antimicrobials in animal production has resulted in increased pressure for the selection of resistant bacterial strains, challenging both animal and human health (4). The objective of this study was to evaluate the antimicrobial resistance profile of *Salmonella* sp. isolated from samples collected from pigs slaughtered in a slaughterhouse in the microregion of Ponte Nova, Zona da Mata, State of Minas Gerais, Brazil.

Materials and Methods

One hundred rectal swabs were collected from pigs slaughtered in a slaughterhouse for the domestic and foreign markets. The samples were isolated, and presumptive identification (2) was performed using morphotintorial and biochemical characteristics using the Rugai kit with Lysine-Newprov® and Triple Sugar Iron (TSI) test. The antimicrobial resistance test was performed on 16 samples of *Salmonella* sp. by disk diffusion method using Mueller-Hinton agar (MH) (3). The antibiotic disks used included: amoxicillin 10µg (AMO), doxycycline 30µg (DOX), norfloxacin 10µg (NOR), nitrofurantoin 300µg (NIT), azithromycin 15µg (AZI), tetracycline 30µg (TET), sulfazotrim 25µg (SUT), and ciprofloxacin 5µg (CIP). The diameters of the inhibitory halos were measured and interpreted (3). The colonies of *Salmonella* sp. were suspended in brain and heart infusion broth (BHI) and, after overnight growth at 37°C, the swab with the inoculum was spread on the surface of the MH agar and then the discs were placed on the surface. Subsequently, the plates were incubated in an aerobic environment at 37°C for 24h, and the diameters of the inhibition halos were measured with a ruler from the diffusion disc manufacturer.

Results

A total of 87.5% of the samples presented themselves as "multidrug-resistant" (MDR), as they are resistant to four or more groups of antimicrobials (4). SUT, NIT, and AMO were the antimicrobials with the highest number of resistant samples (87.5%), followed by CIP, DOX, NOR, AZI, and TET with 81.25%, 75%, and the remaining 43.75% respectively (Table 1).

Conclusions and Discussion

A high rate of *Salmonella* sp. multidrug-resistant to the tested antimicrobials were found, emphasizing the need for the rational use of antimicrobials and the development of alternative products to these drugs, such as secondary compounds of plants and probiotics, which enable the improvement in the sanitary quality of animal products, reducing the risks of contamination and the selection of multi-resistant bacteria.

Table 1. Antimicrobial resistance profile of 16 samples analyzed.

Antimicrobials tested (n=16)	Resistent (%)	Susceptible (%)
AMO	14(8.,5%)	1(6.25%)
DOX	12(75%)	4(25%)
NOR	7(43.75%)	8(50%)
NIT	14(87.5%)	2(12.5%)
AZI	7(43.75%)	9(56.25%)
TET	7(43.75%)	3(18.75%)
SUT	14(87.5%)	1(6.25%)
CIP	13(81.25%)	1(6.25%)

The remaining samples (n = 16) were intermediate in terms of susceptibility to the tested antibiotics.

Acknowledgments

The authors thank Cefar Diagnóstica LTDA, São Paulo, SP, Brazil.

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Detection of multidrug-resistant *Staphylococcus aureus* in pig farms located in the Rio de Janeiro state, Brazil

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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a multidrug-resistant (MDR) bacterium that cause various infectious diseases in humans. Due to its threat to public health, the World Health Organization included it in a list of agents that should be prioritized for research on new antibiotics[1]. *S. aureus* may also colonize healthy humans and other animals. It has been recovered from animals and their human contacts, suggesting a transmission between different host species[2]. Pork is one of the most consumed meat worldwide. Brazil is the fourth largest pork producer and exporter[3]. Pigs colonized with MDR *S. aureus* may represent a risk to farm employees and pork consumers. Scarce data on antimicrobial resistance of *S. aureus* strains isolated from pig farms in Brazil are available. Thus, this study evaluated the occurrence of pigs and farm employees colonized with MRSA in farms located in Rio de Janeiro state.

Material and Methods

Nasal swabs were collected from 250 pigs and 28 farm employees from 16 and nine farms, respectively, located in different cities of the Rio de Janeiro state, from 2014 to 2019. Isolation was performed on Mannitol Salt Agar, with and without oxacillin. Selected colonies were identified by MALDI-TOF. Antimicrobial resistance was evaluated by the disk diffusion method for 11 antimicrobial agents. Detection of the *mec*A gene by PCR was used to confirm MRSA identification. Isolates showing resistance to ≥ 3 antibacterial agents were classified as MDR.

Results

We isolated *S. aureus* from 15 (6%) pigs and seven (25%) humans from eight and four farms, respectively. MRSA strains were exclusively isolated from animals (n=3; 1.2%). Other MDR strains were detected in pigs (n=5; 2.0%) and their human contacts (n=5; 17.9%) in different farms. MDR strains were resistant to four to seven antimicrobial agents. The highest resistance frequencies were observed to penicillin (pig: 53.3%; human: 85.7%) and tetracycline (pig: 53.3%; human: 85.7%), followed by clindamycin (pig: 60%; human: 71.4%) and erythromycin (pig: 60%; human: 57.1%). Resistance was also observed to chloramphenicol (pig: 40%; human: 28.6%), ciprofloxacin (pig: 46.7%; human: 42.9%), gentamycin (pig: 20%; human: 28.6%), oxacillin (pig: 20%) and sulfamethoxazole-trimethoprim (pig: 33.3%). Induced resistance to clindamycin was only observed in human isolates. MDR isolates were identified in 53.3% (MRSA: 20%; other MDR strains: 33.3%) of the pigs and 71.4% of the humans colonized with *S. aureus*. In two farms, the MDR strains isolated from pigs and humans shared the same resistance patterns.

Discussion and Conclusions

The number of pigs investigated was higher than that of farm employees, but *S. aureus* colonization prevalence was lower among animals. This observation may suggest a lower adaptability of this bacterial species to the pigs. The colonization prevalence of pig farm employees was similar to the general colonization prevalence of the human population[4]. MRSA colonization frequencies is usually low in the swine population[1]. However, highest frequencies have been reported in some European countries due mainly to a specific genetic lineage (CC398)[5]. This lineage was also detected among the pig isolates of our study (data reported in another scientific meeting). Other MDR strains were isolated from animals and their human contacts with strains sharing the same resistance pattern. Molecular analyses may confirm whether the strains belong to the same genetic lineage, which would suggest inter-species transmission. Of note, we observed resistance to several antimicrobial agents prescribed to treat human and animal infections. Colonization of food-producing animals with drug-resistant bacteria may affect food security and, therefore, the continuous monitoring of antimicrobial resistance among bacteria isolated from animals is required to avoid the spread of MDR bacteria.

Acknowledgments

FAPERJ, CNPQ

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Ethyl acetate extraction of *Lactobacillus plantarum* crude metabolites protect intestinal tissue exposed to deoxynivalenol in pigs: histopathological findings

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Introduction

Deoxynivalenol (DON) is a type B-trichothecene mycotoxin produced by plant fungi from *Fusarium* species (1). Cereal grains are widely contaminated with DON and animals, particularly pigs, are exposed to its toxic effects (2). DON is not destroyed in feed processing , thus strategies to mitigate toxicity are of increasing interest. Detoxification methods include feed additives such as clays and bacterial biotransformation. Acid lactic bacteria are able to minimize mycotoxin toxicity and a better understanding about the mechanisms involved can contribute to the development of decontamination strategies. The aim of this study was to evaluate the effects of *Lactobacillus plantarum* metabolites in intestinal explants expoded to DON (3, 4).

Materials and Methods

Crude cell free supernatants were obtained from two strains of *Lactobacillus plantarum* (Lp) culture, one a comercial strain (ATCC 14917) (strain 1) and the other isolated from wheat grain (strain 2). Both Lp culture supernatant were followed by extration in water immiscible solvents dichloromethane and ethyl acetate to obtain the bacteria metabolites. Intestinal explants were obtained from the jejunum of four 24-days old piglets. Firstly, the explants were incubated with the bacteria metabolites for two hours; posteriorly the explants were exposed to DON for one hour. After incubation, explants were processed for histological assay. An histological lesion score was established to quantify the intestinal changes. The explants were exposed to the following treatments: culture medium (control), DON (10µM), bacteria metabolites of both strain of Lp extracted with dichloromethane and ethyl acetate alone or associated with DON.

Results

DON induced significant intestinal lesions as apical necrosis, decreased villi height, altered enterocyte morphology compared to control (p=0,0005). Meanwhile, explants treated only with Lp metabolites extracted with ethyl acetate of both culture supernatants (strain 1 and strain 2) showed increased villi height, preserved columnar epithelial morphology, reduction of apical necrosis and tight junctions integrity when compared to DON treated explants. The lesion score in these groups remained similar to control maintaining intestinal integrity (p=0.008, p=0.002 respectively). When exposed to DON a significant reduction in morphological lesions was also achieved (p=0,02) in explants treated specifically with crude metabolites of strain 2 extracted with ethyl acetate.

Conclusions and Discussion

This results demonstrated that ethyl acetate extraction of *L. plantarum* metabolites improve intestinal integrity/morphology when exposed to DON and could represent a suitable protection and a solution against fungal degraded stuff.

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Ultrastructural analysis in piglets intestinal explants exposed to *Lactobacillus plantarum* crude metabolites and deoxynivalenol

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Introduction

Deoxynivalenol (DON) is a type B-trichothecene mycotoxin produced by plant fungi from *Fusarium* species (1). Cereal grains are widely contaminated with DON; therefore food and feed are exposed to be contaminated (2). Since, this mycotoxin is partially destroyed in food processing during the high temperature stage, detoxification methods should be applied to mitigate animal intestinal damages. Biotransformation of mycotoxins and preservation of intestinal tissue by acid lactic bacteria is already a way to minimize the deleterious effects of this toxin (3)

Materials and Methods

Crude cell free supernatant were obtained from a native strain of *Lactobacillus plantarum* (Lp) culture in wheat. Lp culture supernatant were followed by extration in water immiscible solvent ethyl acetate to obtain the bacteria metabolites. Intestinal explants were obtained from jejunum of four animals and incubated with the bacteria metabolites for two hours; explants were exposed to DON one hour after the metabolites. After incubation, explants were process for transmission electron microscopy observation. For this, samples were fixed with 2% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) at room temperature for 20 h. The samples were then washed with sodium cacodylate buffer (0.1 M, pH 7.2) and treated with 1% osmium tetroxide in sodium cacodylate buffer for 1 h, subjected to gradual dehydration in ethanol (70, 80, 90 and 100%). After, samples were embedded in epoxy resin and maintained for 3 days at 60 °C for polymerization. Ultrathin sections were stained with uranyl acetate and lead citrate and analyzed by JEOL JEM - 1400.

Intestinal explants from piglets were exposed to the following treatments: culture medium (control), DON (10µM), bacteria metabolites of Lp extracted with ethyl acetate alone or associated with DON.

Results

DON induced significant intestinal lesions. Enterocytes presented increased intercellular spaces, decreased or absent microvilli and junction complexes, and presence of apoptotic corpuscles. Meanwhile, explants treated only with metabolites extracted with ethyl acetate showed a continuous monolayer of enterocytes with preserved microvilli with evident intercellular junctions, cytoplasm was homogenous and goblet cells were observed between enterocytes with secretory granules, remaining similar to control.

Conclusions and Discussion

The morphologic intestinal changes were clearly observed by transmission electron microscopy analysis. Enterocyte morphology was improved by ethyl acetate extraction of *L. plantarum* metabolites and could represent a suitable protection and a solution against fungal degraded stuff.

Acknowledgments

Electronic Microscopy Center, State University of Maringa.

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Evaluation of the effectiveness of antibiotics combination for the control of porcine respiratory complex (PRC) pathogens in an experimental challenge

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Introduction

In pig production, the use of antibiotics varies between individual farms which may, at least partly, be related to farm characteristics, such as technical and economic performance. In an attempt to stop the increase or even reduce antimicrobial resistance in zoonotic pathogens, the European Commission has prohibited the use of antibiotic feed additives as growth promoters in pig production (1). The correct use (therapeutic) of antibiotics is necessary in pig production but unnecessarily there is a combination of antibiotics (mix) many times antagonists between them. The aim of this study was demonstrated use antibiotics according to label indications are effectives vs PRC agents compare with use mix of antibiotics.

Materials and Methods

7 groups were formed and received antibiotics by feed. Negative control (NC, No antibiotics & No challenge)= 5 pigs, Positive control (PC, challenged without antibiotics)= 5 pigs, Tx1 Florfenicol (40 ppm) + Tylosin (80 ppm)= 12 pigs, Tx2 Tiamulin (110 ppm) + chlortetracycline (300 ppm)= 12 pigs, Tx3 Doxycycline (250 ppm)=12 pigs, Tx4= Lyncomicyn + Spectinomycin (110 ppm)= 12 pigs, Tx5 (mix) Tiamulin (100 ppm) + chlortetracycline (300 ppm) + Colistin (1000 MIU, Millons of international units)= 12 pigs. Piglets with 12 hours were weaned from sows (PRRS & *Mycoplasma hyopneumoniae* negatives) and received in isolations rooms. From 12 hours to 20 days of age, piglets received special feed + artificial milk in order to reduce mortality and increase daily gain then when piglets reach 21 days and until 49 days all groups started to eat inside feed different antibiotics according their treatment, then at 35 days of age, all groups were challenged with a doses of $1X10^8$ for each bacteria as: *Actinobacillus pleuropneumononiae (APP), Pasteurella multocida (Pm), Haemophilus parasuis (Haem), Bordetella bronchisepticum, Streptococcus suis & Actinobacillus suis (Act suis);* intranasal via. The only group didn´t receive challenge was NC. At 49 days all piglets were euthanized according to INIFAP procedures and gross lesions were recorded and histopathology (upper respiratory tract) & qPCR (from lungs) were performed in order to evaluate the best group vs mix group.

Results.

Table 1. Average (%) of gross lesions by group

	EI	En	Hid	Per	Fib	DM	Hem
Tx1	100	0.0	0.0	0.0	0.0	0.0	0.0
Tx2	80	10	10	10	0.0	0.0	0.0
Tx3	90	40	20	0	0.0	0.0	0.0
Tx4	57	14	43	14	14	29	0
Tx5	86	0.0	0.0	0.0	14	0.0	0.0
PC	40	0.0	60	40	0.0	12	40
NC	80	0.0	60	40	0.0	12	0.0

EI= Interlobular edema, En= Enfisem, Hid= Hidropericardium, Per= Pericarditis, Fib= Fibrine in lungs, LD= % Lung damage, Hem= Hemoptisis.

Table 2. Percentaje of microscopic lesion by group.

	PC	NC	Tx1	Tx2	Tx3	Tx4	Tx5
NIL	40	40	20	20	0.0	80	0.0
HIN	60	60	100	80	0.0	0.0	100
IE	40	20	80	0.0	100	0.0	0.0

NIL= Limphoplasmocitic intersticial edema, HIN= Histiocitic intersticial neumonia, IE= Interlobular oedema.

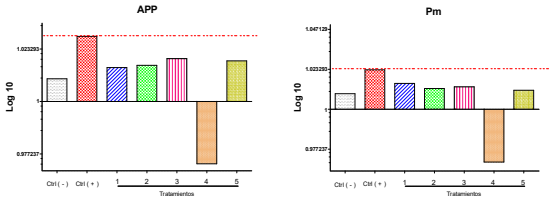

Figure 1. Results from PCR for APP and P. multocida

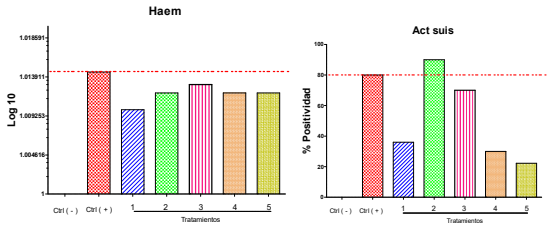

Figure 2. Results from PCR for *H. parasuis* and *A. suis*

Conclusions and Discussion

Its no possible to identified which groups is the most effective in order to reduce gross (G) and microscopic (M) lesions and decrease cuantity of respiratory bacterias but NC and PC had more G & M lesions and cuantity of bacterias by PCR. Comparative results between Tx2 & Tx5; Tx2 had better results in all variables reviewed. The treatment with Tiamulin + chlortetracycline is the best option, in order to control respiratory agents.

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The use of probiotics in gestation and lactation to increase the weight at weaning

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Introduction

The ban on the use of antibiotics as a growth promoter has stimulated the study of probiotics in animal feed, however, most research occurs in the growing and finishing phases. This study was carried out during gestation and lactation and was designed to evaluate the use of commercial probiotics in these stages of production.

Materials and Methods

Fifteen primiparous sows of the same lineage were transferred to the conventional farrowing house of the farm, five days before farrowing. The experimental design was completely randomized, with three treatments and five repetitions that were provided from 40 days of gestation until weaning. The treatments were T1: Control (diet - according to Rostagno, 2017), T2: Basal diet + probiotic 1kg/ton, T3: Basal diet + probiotic 2kg/ton. The commercial mix Nutri+porcine® consisted of lysine, methionine, glucan, mannan, *Bifidobacterium difidum, Saccharomyces cerevisiae, Enterococcus faecium, Bacillus subtilis, Lactobacillus acidofhilus, L. lactis, L. casei.* At birth, piglets were identified and weighed. Were evaluated: feed intake of sows, colostrum quality, number of piglets born alive, stillborn and mummified, weight gain and mortality.

Results

Regarding sows, primiparous of the treatment 3 consumed a larger amount of feed than sows of other treatments. The diets provided did not influence the variables analyzed for colostrum (Table 1).

Table 1. Colostrum analysis (Amount of fat, lactose, total solids, casein and total protein) and average daily feed intake of sows per treatment.

Treatment	Fat (%)	Lactose (%)	Total Solids (%)	Casein (%)	Total Protein (%)	ADFI (Kg)
1	5,15	2,67	20,62	10,01	11,69	5,56[b]
2	5,27	1,97	22,4	13,63	13,63	6,01[ab]
3	5,31	2,41	21,28	12,49	12,49	6.43[a]

ADFI: Average Daily Feed Intake. Different letters represent significant differences at $P < 0.05$.

In piglets there was a significant difference between treatments, and the best rates of weaning weight and daily weight gain (DWG) were observed in the animals of treatment 3. Piglets born to females fed with a high dose of probiotic were approximately 900g heavier than those born to females who did not receive the product. The rates of born alive, stillbirths, mummified and mortality were not influenced and are within the acceptable rates for the phase. (Table 2).

Table 2. Weight at birth and weaning, averages of daily weight gain and born alive piglets, and percentage of mummified, stillborn and mortality by treatment.

Treat.	Weight (g)		Average		%		
	Birth	Weaning	DWG (g)	BA	MM	S	M
1	1261,035	7005,26[b]	194,24[b]	13,2	1,52	3,03	4,55
2	1291,035	7275[b]	198,42[b]	13,5	2,96	2,22	1,48
3	1148,69	7948,14[a]	226,20[a]	13,7	1,46	0,00	2,19

DWG: Daily Weight Gain, BA: Born alive, MM mummified, S: Stillborn, M: Mortality.
Different letters represent significant differences at $P < 0.05$.

Discussion and Conclusions

The administration of the probiotic during gestation and lactation increased the feed intake of lactating sows, which is favorable because this phase is characterized by high nutritional requirements and low food consumption. The probiotic did not influence the quality of colostrum, but it had a positive influence on weaning weight. It is known that the weight gain of the litter is related to the production or the quality of the milk (2). The fact that the quality of the colostrum did not change, together with the better weight gain of the piglets are indicative that the increase in feed consumption, caused by the probiotic activity of the diet, resulted in an increase in milk production by the sows and, consequently, in the best weight at weaning. It is known that the weaning weight directly influences the performance of the piglets in the following stages of production. So the results obtained in this study indicate that the supply of probiotics for gestation and lactation sows is a viable alternative in pig farming.

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BACTERIAL DISEASES

Glasser's Disease – Case report

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Introduction

Glasser's Disease is a disease of high relevance in pig farming, caused by *Haemophilus parasuis*, which can result in major economic damage to the producer. It is a disease with a high mortality rate, usually presenting in the form of outbreaks. This work aims to report Glasser's disease in a multi-farm system, as well as raise awareness about the importance of the disease in the sanitary and economic aspect.

Case Report

In a pig farm, one of the nursery barn was observed that some piglets, newly weaned, between 35 and 55 days of age, fed with initial ration, presented respiratory disease symptomatology, such as apathy (Figure 1), rough hair, productive cough, ocular secretion, intense respiratory difficulty and fall-behind. In the necropsy, was observed polyserositis (Figure 2), peritonitis, pericarditis, hepatization of the middle and cranial lobes and some pleuritis and adhesions in the abdominal and thoracic cavity. For a definitive diagnosis, samples of the sick animals were collected for bacteriological examination, and fragments of the heart and lung were collected, in addition to pleura and joint swabs. The culture result of the sent material was positive for *Haemophilus parasuis* serotype 5 in lung, heart and pleura samples. In the antibiogram, the bacteria was sensitive to 11 of the 12 antibiotics tested, including Ceftiofur and the tetracycline group. For treatment, was administered Doxycycline 50%, at the dose of 200ppm/1000kg of feed, only for this phase, for seven days, for the whole group, replacing the Amoxicillin which was the prophylactic antibiotic used previously, as well as the application of injectable Ceftiofur, at the dose of 5mg/kg, for animals that still presented symptoms. In addition, measures were taken to reduce the main predisposing factors, avoiding the stress problems that animals of this phase are often submitted.

Results

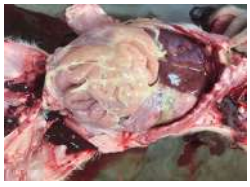
After the treatment period the animals fully recovered. As prophylaxis, antibiotics continue to be administered by feed, in pulses and in strategic phases, using Doxycycline.

Conclusions and Discussion

The animals of this report initiated the symptomatology of the disease in the phase of 35-55 days of age, recently weaned in the nursery barn, being according to the literature that affirms the disease mainly affects this phase[4]. Among the pathological findings of this report, was observed the presence of polyserositis, macroscopic lesions suggestive of the acute phase of infection[1]. In addition adhesions and pleuritis present in chronic cases, according to that described in the literature. The diagnosis of the disease was obtained through the association of clinical examination, findings of necropsy and confirmatory through culture and antibiogram, as recommended in the literature[3]. Being the conclusive diagnosis, the isolation of the agent through tissue fragments of the affected animals also described by some authors. In this report, as prophylaxis, measures were adopted to reduce the main predisposing factors related to animal stress[2]. Different from that suggested by the literature, in the studied property the vaccine was not used as a method of disease control, even though it was considered one of the most appropriate prophylactic measures for producers. Thus, intensive work of veterinarians is necessary, both in control ling and awareness about the prophylaxis of the disease, since there is an exponential increase in bacterial agent resistance to antibiotics, which can make vaccination a viable alternative in relation to cost-benefit.

Figure 1. Apathetic piglet presenting swollen joints

Figure 2. Polyserositis with presence of fibrinous content and adhesions



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Comparative analysis using pulsed-field gel electrophoresis (PFGE) highlights potential transmission of *Salmonella* spp. strains between pigs in a farm from São Paulo State, Brazil

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Introduction

Salmonella spp. are gram-negative bacteria, responsible for economic losses in livestock animals, as well as being a zoonotic agent linked to foodborne disease (1). There are two species (*S.* bongori and *S.* enterica), subdivided into six subspecies (*arizonae*, *diarizonae*, *enterica*, *houtenae*, *indica* and *salamae*), with more than 2.500 serotypes. The infection of warm-blood animal species, including pigs, is caused by serotypes of *S. enterica* subspecies *enterica* (2). The aim of the study was to investigate the occurrence of *Salmonella* spp. strains in fecal samples of pigs, as well as analyzing the pulsed-field gel electrophoresis (PFGE) patterns of these strains, to identify potential transmission between pigs.

Materials and Methods

Thirty-two rectal swab samples from pigs were collected from a property located in São Paulo State, Brazil. To isolate *Salmonella* spp., rectal swabs were enriched in selenite cystine, Muller-Kauffmann tetrationate and Rappaport-Vassiliadis broths, and incubated (37°C, 24h). The broths were then seeded on plates containing modified brilliant green agar and xylose lysine tergitol agar and incubated (37°C, 24h). Colonies with morphologic characteristics of the genus Salmonella (3) were inoculated in tubes containing triple sugar iron and lysine iron agar, followed by incubation (37°C, 24h). After biochemical confirmation, slide agglutination tests were performed using somatic and flagellar polyvalent *Salmonella* antisera. Positive samples were sent to the laboratory for serotyping. Also, positive samples were subtyped by a standardized rapid pulsed-field gel electrophoresis protocol (4).

Results and Discussion

Out of the 32 rectal swab samples collected, 19 (59.4%) were positive for *Salmonella* genus. From positive samples, 3 (15.8%) were positive for *S.* Agona, 14 (73.7%) for *S.* Senftenberg and 2 (10.5%) for *S.* Schwarzengrund. *Salmonella* genus was found in all categories of pigs, being positive: 3/5 pregnant sows (60%), 4/5 lactating sows (80%) and 3/8 empty sows (37.5%), totalizing 10/18 (55.6%) sows; 2/3 boars (66.7%); 7/11 piglets (63.6%). PFGE analysis identified 2 profiles (profile 1, 2) of *S.* Agona, one profile (profile 3) of *S.* Senftenberg and one profile (profile 4) of *S.* Schwarzengrund. Profile 1 of *S.* Agona revealed a clonal identity between the isolates found in two categories (pregnant and empty sow), showing possible transmission between them. Profile 3, isolated for *S.* Senftenberg, revealed a clonal identity between the isolates found in sows, boars and piglets, showing a possible transmission of this serotype between all categories. Profile 4, isolated for *S.* Schwarzengrund, revealed a clonal identity between the isolates found in lactating sows, showing possible transmission between them (Table 1).

Table 1. Profiles of *S.* Agona, *S.* Senftenberg and *S.* Schwarzengrund isolated in pigs using PFGE analysis.

Serotypes	Profile	Animal	Category
S. Agona	1	1	pregnant sow
		2	empty sow
	2	3	piglet
S. Senftenberg	3	4	pregnant sow
		5	pregnant sow
		6	lactating sow
		7	lactating sow
		8	empty sow
		9	empty sow
		10	boar
		11	boar
		12	piglet
		13	piglet
		14	piglet
		15	piglet
		16	piglet
		17	piglet
S. Schwarzengrund	4	18	lactating sow
		19	lactating sow

Conclusions

The isolation of different types of *Salmonella* strains in pigs linked to PFGE analysis allowed us to conclude that the bacteria has spread between the various categories of animals in the farm studied.

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Effect of the supplementation of a yeast derived purified β-glucan on productive performance and lesion incidence of piglets challenged with *Mycoplasma hyopneumoniae*

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Introduction

Mycoplasma hyopneumoniae (Mh) is the etiologic agent of Enzootic Pneumonia (EP) in swine. This is a chronic disease in pigs that presents a high prevalence (38% to 100%) in almost every high swine producing area of the world. Moreover, EP causes significant economic losses in swine production. Thus, it is important to develop prevention programs other than the use of antibiotics. Some prebiotics derived from yeast *Saccharomyces cerevisiae* such as β-glucans have shown immunomodulatory activities along with regenerative effects at cellular level. These two benefits of β-glucans make them an interesting option for disease prevention in swine. Thus, an experiment was conducted to evaluate the effect of a *Saccharomyces cerevisiae* 50% purified product in pigs under a *Mh* challenge.

Materials and Methods

Thirty two newly weaned piglets with an average initial weight of 6 kg were randomly assigned to one of 3 treatments: Negative Control (without challenge), Positive Control (Challenged), and Positive Control + β-glucan (Safglucan 250 g/ton of feed). A diet containing the nutritional requirements for the prestarter phase was offered *ad libitum.* Treatment was implemented from week 1 to week 3 of the experiment and in the beginning of week 4, the *Mycoplasma hyopneumoniae* challenge was conducted in an isolated aerosol chamber. Animals were monitored daily for typical EP signs. All the animals were euthanized at the end of the experiment and blood samples were prepared for conducting blood testing and assessing interleukin levels of TNF-α, IL-1β and IL-6. Also nasal swabs were collected to perform PCR analysis to detect *Mh*. Finally, productive performance was evaluated using average daily gain (ADG) as the indicator.

Results

In the β-glucan supplemented group the samples for *M. hyopneumoniae* antibodies detection were negative because of the fact that the proportion M/P was smaller than the reference limit of 0.400. The lungs of piglets in the Negative Control presented a very small incidence of lesions caused by EP (0.4% of the surface in the apical pulmonary lobe) while in the lungs obtained from the piglets in the Positive Control Group the lesion incidence was greater (14.43%). In the β-glucan supplemented group the lung lesion incidence was minimal (0.51%).

The ADG of the Positive Control group was the lowest. The β-glucan supplemented group presented an intermediate ADG while the Negative Control had the highest ADG during the experiment (Table 1).

Table 1. Final weight of piglets challenged with Mycoplasma hyopneumoniae.

Treatment	Control Neg	Positive Control	β-glucan
Final weight	22.8 [a]	13.0 [c]	17.0 [b]
Std deviation	0.73	0.39	1.32

[a,b,c] Different letters in the same row means significant difference between the treatments (P<0.05)

No significant differences among treatments were detected for any of the evaluated interleukins (TNF-α, IL-1β y IL-6).

Discussion

In this experiment an average reduction of 96% in the lung lesions was observed due to β-glucan supplementation. As expected *Mycoplasma hyopneumoniae* challenge reduced ADG. However, β-glucan supplementation had a positive impact in ADG probably due to its immunomodulatory effect when animals were exposed to the challenge. Despite the improvement in productive performance, no significant differences were detected in the cytokine (TNF-α, IL-1β and IL-6) concentrations due to high individual variability in the response. Probably a larger number of repetitions is required for a more precise assessment of cytokine response.

Conclusions

The lower lung lesion incidence observed in the β-glucan supplemented piglets allows us to conclude that the dosage utilized of the product in this experiment was effective. Moreover, the improvement in productive performance also showed potential positive benefits of β-glucan supplementation when animals are exposed to a *Mycoplasma hyopneumoniae* infection.

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Key words: *Mycoplasma hyopneumoniae*, β-glucan, Immunomodulatin, Inflamation

Detection of *E. coli* from feces and oral fluid samples through culture and qPCR: a warning for virulent and resistant strains in the swine production

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Introduction

Escherichia coli is a Gram-negative bacterium of the family Enterobacteriaceae that can be related to several diseases in swine. Consequently, they may cause great economic losses. Some pathotypes are important for the swine industry, such as Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC) and Shiga toxin-producing *E. coli* (STEC). These groups have different virulence factors that can be used for detection in biological samples[1]. In this study, virulence factors of *E.coli* were detected through qPCR of isolates from feces and oral fluid of weaned pigs. The resistance to antimicrobials was also described.

Materials and Methods

Feces and oral fluid from 50 healthy weaned pigs were obtained at five different farms in the state of São Paulo, Brazil. We randomly chose five animals from each pen, and in each farm two pens were used in the experiment. The suspected isolates were recovered from MacConkey Agar after a pre-enrichment step, and confirmed by biochemical tests using the API 20E Microbial Identification kit (bioMérieux®). Following, the antimicrobial resistance was tested with Polisensidisc 15 (DME®) in Mueller-Hinton Agar. Genomic DNA was extracted using a NewGene Prep and NewGene Preamp kits on the samples after the pre-enrichment step. The Real-time PCR (qPCR) was performed to check the presence of coding genes for virulence factors (Sta, Stb, Lt, Stx1, Stx2 and Stx2α). These reactions were performed on a Step one Plus™ equipment (Applied Biosystems®), using EXOone qPCR Kit (Exopol®).

Results and Discussion

A total of eighteen isolates were obtained from the cultures and submitted to antimicrobial resistance testing. Among these eighteen isolates, nine were detected in feces samples, eight in oral fluids and one was isolated from the environment using the boot sock method. All five farms had positive samples: Farm 1 (3); Farm 2 (3); Farm 3 (2); Farm 4 (3); Farm 5 (7). The results of antibiotic resistance were: Amikacin (11%); Amoxicillin/Clavulanic Acid (11%); Ampicillin (50%); Aztreonam (66%); Cefazolin Parenteral (50%); Cefazolin Oral (16%); Cefepime (16%); Cefoxitin (22%); Ceftadizime (27%); Ceftriaxone (16%); Ciprofloxacin (61%); Chloramphenicol (33%); Gentamicin (27%); Meropenem (5%); Trimethroprim/Sulfamethoxazole (50%) and Tetracycline (94%). Most antibiotics are commonly used in the swine production and many have shown resistance in other studies[2]. We also had one strain resistant to Meropenem. This antimicrobial is used for severe infections in humans, which reinforces the necessity of prudent use of antimicrobials in the swine production[3]. In addition, real-time PCR results indicated that all five farms had strains positive to, at least, one virulence factor (Table 1). Sta and Stb genes were the most frequent virulence factors found in the samples.

Table 1. Virulence factors tested through qPCR.

Farm	Pen	Sta	Stb	Lt	Stx1	Stx2	Stx2α
1	1	+	+	-	-	+	+
	2	+	+	-	-	+	+
2	1	+	+	+	-	-	+
	2	+	+	-	-	-	+
3	1	+	+	-	-	-	+
	2	+	+	-	-	-	-
4	1	-	+	-	-	-	-
	2	+	-	-	-	-	-
5	1	-	-	-	-	-	+
	2	-	-	-	-	-	-

Conclusion

The emergence of resistant and virulent *E. coli* can be a difficult problem to solve in the swine production, and can open doors to more severe infections. This study raises a red flag on indiscriminate use of antibiotics, since some strains were very resistant, even for antimicrobials that are very important in human medicine.

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First isolation and whole-genome sequencing of a *Shewanella algae* strain using the boot sock method at a swine farm in Brazil

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Introduction

Shewanella spp. are Gram-negative bacteria mostly found in marine environments. Although infections in humans are considered rare, several studies have reported *Shewanella putrefaciens* as a cause of severe illnesses, such as endocarditis, tissue necrosis and peritonitis. *Shewanella algae* seems to be more virulent than other species of this genus[1]. In this study, we report for the first time an unexpected isolation and identification of *Shewanella algae* using the boot sock method at a swine farm in Brazil. The genome sequence and the antimicrobial susceptibility of this strain were also described in this study.

Materials and Methods

The sample was collected using disposable shoe covers from a pen of weaned pigs at a farm in the State of São Paulo, Brazil. The boot sock method consists in dragging the boots all over the area to be tested. The primary aim of this study included the isolation of *Salmonella enterica*, thus, the methodology was based on an enrichment step with Tetrathionate, Selenite Cystine and Rappaport Vassiliadis Soya broths for 48 hours, followed by plating on Hektoen Enteric, Salmonella Shigella, MacConkey, Brilliant green, XLD and XTL-4 agar plates. All the enrichment broths were supplemented with a solution of Novobiocin (40mg/µl) [2]. Biochemical identification was performed using the API 20E kit (bioMérieux®), and the antimicrobial susceptibility was tested for 15 formulas using the Polisensidisc 15 kit (DME®). The sequencing data was processed by Fastp. Then, it was input to SPAdes in order to assemble the bacterial genome. Annotation was carrided out with Prokka, and the detection of genes related to virulence and antimicrobial resistance was performed with Abricate, using the databases "The virulence factor database" - VFDB and ResFinder. Genome alignment was done using Quast, and phylogeny was obtained in phylogeny.fr.

Results and discussion

For the first time, this study reports the isolation of *Shewanella algae* from a pen of weaned pigs at a farm in the State of São Paulo, following a methodology designed for the isolation of *Salmonella enterica*. *Shewanella* spp. may be recovered in specific media for Enterobacteriaceae and can be misidentified as *Salmonella enterica*[3]. The strain was first identified as *Shewanella putrefaciens* by the API 20E kit. However, after whole-genome sequencing, it was identified as *Shewanella algae*, based on the total genome alignment, as well as on 16S ribossomal phylogeny. In addition, this strain was resistant to 46.66% (7 out of 15) antimicrobials tested: Amoxycilin, Clavulanate, Ampicilin, Cefazolin, Ciprofloxacin, Chloramphenicol and Trimethroprim-Sulfamethoxazole, which are common formulas used in the swine production as in-feed or therapeutic[4].

The draft-genome (286 contigs >200 bases, N50 = 88.103 bases) has 4.953.873 bases, and 4.531 genes were detected. A total of 11 genes found in the draft-genome were similar to virulence factors already described. It was detected 26 genes similar to antimicrobial resistance genes, which supports the prediction that this strain could be resistant to 21 antibiotics: Amoxicillin, Ampicillin, Streptomycin, Trimethoprim, Amikacin, Sulfamethoxazole, Chloramphenicol, Ciprofloxacin, Doxycycline, Cephalothin, Piperacillin, Ticarcillin, Tobramycin, Gentamicin, Ampicillin + Clavulanic acid, Amoxicillin + Clavulanic acid, Tigecycline, Imipenem, Florfenicol, Tetracycline and Minocycline. This wide potential ability to be resistant to antimicrobials could mean that this is a multidrug-resistant bacteria.

Conclusion

This study reports the first isolation of *Shewanella algae* using the boot sock method at a swine farm in Brazil. The whole-genome sequencing was important to identify the strain correctly, suggesting that it has some virulence characteristics and that it could be a multidrug resistant strain. In summary, our data can improve the understanding of the epidemiology and taxonomy of these bacteria.

Acknowledgments

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PK/PD and clinical relationships of Pharmasin® 250mg/g Premix (tylosin phosphate) administered to pigs for the treatment of ileitis caused by *Lawsonia intracellularis*

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Introduction

Lawsonia intracellularis (*Li*) is an anaerobic obligate intracellular bacterium infecting the small intestine and infrequently also the large intestine in pigs (3).
Pharmasin® is a macrolide antibiotic registered for the treatment and prevention of Porcine Intestinal Adenomatosis (PIA) associated with *Lawsonia intracellularis* and of Mycoplasma-based infections caused by *Mycoplasma hyopneumoniae and Mycoplasma hyorhinis.*
The objective of the work was to compare the pharmacokinetics (PK) of the tylosin (Pharmasin® - Huvepharma NV) ileum contents concentration (ICC), to relate this to intracellular MICs (iMICs) against *Li* strains derived from laboratory studies (pharmacodynamics – PD) and evaluate the clinical efficacy of the drug when administered in an artificial infection study.

Materials and Methods

Pharmacokinetics: The tylosin (TYL) ICC and colon contents concentration (CCC) were determined in a pharmacokinetic study with mixed breed pigs (bodyweight 20-25kg) (2). Ten animals (equally male and female) were medicated with tylosin phosphate (Pharmasin® 250mg/g Premix) at 10mg/kg bw/24h via feed for 5 consecutive days ad libitum. *Pharmacodynamics:* The iMICs were derived from studies in which *Li* isolates from Brazil (two isolates) and Thailand (three isolates) grown in McCoy murine fibroblast-like cell cultures were tested (6). *Challenge study:* Tylosin phosphate was used in an artificial challenge study at 40ppm (commencing 4 days pre-challenge, continuing for 16 days after infection (D-4-D16), when the dose was reduced to 20ppm, D17-D28) and at 100ppm (commencing 7 days after challenge, medication 21 days, D7-D28)) (4). *Li* challenge strain LR 189/5/83: iMIC = 1.0 / extracellular MIC =16 (5).

Results

Pharmacokinetics: The tylosin ICC was recorded at 31.4 µg/g at a dose of 10mg/kg bw. CCC of tylosin was 89.4 µg/g. Tylosin ICC at treatment dosage (5mg TYL/kg bw) is estimated to be around 16 µg/g (15.7 µg/g) which is in line with tylosin ileum PK calculations previously published (1). *Pharmacodynamics:* The TYL iMIC range for the three Thailand *Li* strains was 2-16 µg/ml; for the two Brazil *Li* strains an iMIC range of 2-8 µg/ml was measured. The determined iMIC values indicate that tylosin was capable of inhibiting the growth of *Li* in the in vitro cultured enterocytes. *Challenge study:* In the study none of the pigs on the tylosin prevention and treatment programmes showed any gross or histopathological signs of ileitis infection. In the infected untreated group macroscopic lesions in 5/8 pigs (62.5%) and microscopic lesions in 7/8 pigs (87.5%) were found in the ileum and in 3/8 pigs (37.5%) in the caecum.

Conclusions and Discussion

The pharmacokinetic properties of tylosin are characterized by low intestinal absorption and high penetration plus accumulation into gut enterocytes. Effective tylosin concentrations are achieved in the ileum contents by infeed medication. These are sufficient to inhibit the development of ileitis (40ppm) and able to treat ileitis infections effectively when given at 100ppm (5mg/kg bw). The use of PK/PD relationships is an effective model in predicting the efficacy of tylosin phosphate to inhibit *Lawsonia intracellularis* and to treat ileitis infections in pigs.

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PK/PD and clinical relationships of tiamulin and lincomycin administered to pigs for the treatment of ileitis caused by *Lawsonia intracellularis*

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Introduction

Vetmulin® (tiamulin) and Lincocin® (lincomycin) are antibiotics registered for the treatment of ileitis infections caused by *Lawsonia intracellularis* (*Li).*

The purpose of the paper is to compare the pharmacokinetics (PK) of tiamulin (Vetmulin® - Huvepharma NV) and lincomycin (Lincocin®) ileum contents concentration (ICC), to relate this to intracellular MICs (iMICs) against *Lawsonia intracellularis* derived from laboratory studies (pharmacodynamics – PD) and evaluate the clinical efficacy of both drugs when administered in artificial infection studies.

Materials and Methods

Pharmacokinetics: The tiamulin (TIA) ICC concentration was estimated using a PK model based on the tiamulin colon contents concentration published following tiamulin feed administration at 110ppm (6.6mg/kg bw) and 220ppm (13.2mg/kg bw) (1). The lincomycin (LIN) ICC concentration was determined in PK experiments using lincomycin infeed concentrations of 110ppm and 220ppm (2).

Pharmacodynamics: The iMICs were derived from studies in which *Li* isolates from USA, Denmark, UK, Brazil, Thailand and Korea grown in McCoy murine fibroblast-like cell cultures were tested (4,5).

Challenge studies: Tiamulin was used in an artificial challenge study at 50ppm (approx. 2.5mg/kg bw, commencing 2 days pre-challenge, continuing for 21 days until trial determination) and at 150ppm (approx. 7.5mg/kg bw, commencing 7 days post-challenge, medication 14 days) (3). *Li* challenge strain LR 189/5/83: iMIC=0.125µg/ml. Lincomycin was tested using an intestinal mucosal homogenate challenge at 44ppm and 110ppm in the feed (21 consecutive days; treatment onset >10% (site A pigs)/>20% (site B pigs) showed abnormal faecal score) (6). The iMIC of the *Li* strain used in this challenge study was not determined.

Results

Pharmacokinetics: The TIA ICC was recorded at 0.82µg/g (110ppm) and at 2.33µg/g (220ppm). Lincomycin ICC concentrations of 10.01 µg/g (110ppm) and 25.05 µg/g (220ppm) were reported.

Pharmacodynamics: The TIA iMIC range against the *Li* strains from USA/Europe was 0.125-0.5 µg/ml and for the Brazil and Asian strains 0.125-2µg/ml. Higher iMICs for LIN were determined for the USA/Europe *Li* strains (8-128µg/ml) and for the *Li* strains from Brazil/Asia (16->128).

Challenge studies: All pigs receiving TIA at 50ppm pre-challenge and 150ppm post-challenge remained clinically normal, free from diarrhoea and showed no gross and histopathological lesions at post mortem. Pigs either at LIN concentration of 44ppm or 110ppm showed lower incidence of clinical impression and of diarrhoea and better performance than the untreated controls. A significantly ($p<0.05$) lower mortality at 110ppm was determined vs. the control.

Conclusions and Discussion

Effective tiamulin concentrations are achieved in the ileum contents by in-feed medication. Low iMICs and narrow MIC ranges confirm the consistent efficacy of tiamulin against *Li* strains generated in important pig production countries worldwide. The tiamulin PK/PD profiles help to explain the high clinical effectiveness of tiamulin seen in ileitis treatment regime at registered dosage (150ppm, 7.5mg/kg bw).

The high and broad range of iMICs verify the low sensitivity of *Li* strains against lincomycin. Due to the lincomycin ileum concentrations achieved at treatment dose and the high MICs, a prediction of the clinical effect of lincomycin in the case of ileitis treatment based on PK/PD relationships cannot be made.

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Antimicrobial resistance, virulence profile and serotype distribution of *Streptococcus suis* isolates recovered from swine in Spain throughout 2018 and 2019

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Introduction

Streptococcus suis is a facultative anaerobic, non motile Gram-positive bacteria from the upper respiratory tract of pigs (1). It is part of the Porcine Respiratory Disease Complex (PRDC), alongside other microorganisms like *Pasteurella multocida*, *Actinobacillus pleuropneumoniae* or Betaarterivirus suid 1 (PRRSV) (2). *S. suis* is one of the major post-weaning bacterial pathogens in the porcine industry (3) and is also considered a zoonotic agent due to its ability to infect humans by contact with ill pigs or contaminated raw pork products (4). It can act as a primary pathogen causing meningitis, septicemia, and arthritis or as an opportunistic agent producing pneumonia (5). Serotypes 2 and 9 are often isolated in diseased European pigs and have shown to be the most virulent ones (6). This work addresses several points regarding *S. suis* pathogenicity, such as serotyping, antimicrobial susceptibility and virulence factor prevalence of 160 spanish isolates.

Materials and Methods

Bacterial isolates were recovered from several swine tissues, such as brain, lungs and joints. A conventional PCR was performed in order to amplify the gene encoding glutamate dehydrogenase (*gdh*), which allows molecular *S. suis* identification. Then, multiplex PCR was carried out in four sets to perform serotyping. Five virulence genes were also studied: *epf, gapdh, luxS, mrp* and *sly*. Each virulence factor was determined by a monoplex PCR with respective primer pairs. Finally, an antimicrobial susceptibility testing (AST) was performed using Bovine/Porcine BOPO6F Vet AST Plates (Sensititre™), which contained eighteen different antibiotics. *S. suis* isolates were cultured 24 hours at 37 ºC in Tryptic Soy Agar + 5% Fetal Bovine Serum. Ten *S. suis* colonies were added to a MilliQ water tube and mixed gently until 0,5 McFarland turbidity standard was reached. 50 µL of the bacterial suspension were transferred to a tube containing 11 mL of Mueller Hinton Broth and 100 µL of the suspension were inoculated to each BOPO6F well. Plates were incubated 24 hours at 37 ºC, and the results were analized using a Sensititre™ Manual Viewbox.

Results

The most prevalent serotypes were 1, 2 and 9. Nevertheless, multiplex PCR allowed the identification of eight additional serotypes: 3, 4, 5, 7, 8, 10, 16 and 17. Most prevalent virulence gene was *luxS* (93.1%), followed by *mrp* (73.6%), *sly* and *gapdh* (both 70.1%). The *mrp* gene was found in 44.8% of the isolates with different size variants observed in electrophoresis. In regard to antibiotic susceptibility, macrolides were those showing the lowest effectiveness: every *S. suis* strain was considered as resistant to one or more antimicrobials of this family. On the other hand, β-lactams showed a great efficacy against *S. suis,* and all the bacterial isolates exhibited sensitivity to one of the β-lactams present in the plate.

Discussion and Conclusions

As expected, serotypes 2 and 9 were present among the most prevalent serotypes; however, serotype 1 was the most prevalent one (21.8%). On the other hand, the results obtained from AST confirm that antimicrobial resistance was dependent on each *S. suis* strain. This current scenario leads us to making urgent a proper use of antibiotics, as well as exploring alternatives to the use of these compounds, in order to preserve public and animal health. In this respect, a recombinant vaccine incorporating proteins expressed by the studied virulence factors could emerge as one of the possible solutions for preventing *S. suis* disease in swine industry and, consequently, for helping to reduce the undue use of antimicrobials.

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Antimicrobial resistance assessment of *Salmonella enterica* isolated from swine in Brazil during a 14 years period

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Introduction

Salmonella enterica is one of the main agents responsible for enteritis, and may even cause septicemia. *Salmonella enterica* also has zoonotic potential and can contaminate carcasses during the slaughter process (1). The intensification of swine production is associated with an increase in bacterial enteric infections and antimicrobial usage; consequently antimicrobial resistance also increased, which is a concern not only for modern pig farming but also for public health (2). The aim of the present study was to assess the antimicrobial resistance of *Salmonella enterica* isolated from pigs from different regions of Brazil over a 14 years period.

Materials and Methods

A total of 406 *S. enterica* isolates were evaluated; these belong to the Laboratory of Swine Health (FMVZ - USP) culture collection. The strains were isolated from 260 swine from 115 herds located in 10 Brazilian states, between 2004 and 2018. Broth microdilution was performed, with a panel of 17 selected antimicrobials, from 10 antimicrobial classes, for determination of the minimal inhibitory concentrations (MIC) (3). *In vitro* resistance rates were identified for the respective antimicrobials (3) and multidrug resistance was determined as described by Schwarz et al. (4).

Results

Only 41 strains (10.1%) were susceptible to all antimicrobial classes tested, while 299 (73.6%) were classified as multidrug-resistant (Table 1). The antimicrobial classes of highest resistance were quinolones (71.9%) followed by beta-lactams (68.7%). In contrast, resistance against meropenem and fosfomycin were not observed among porcine *S. enterica* strains (Table 2).

Table 1. Strain distribution according to number of resistant classes and isolation year – N (%).

Nº resistant classes	Years			Total
	2004 - 2009	2010 - 2014	2015 - 2018	
0	30 (27.0)	1 (1.2)	10 (4.8)	41 (10.1)
1 - 2	35 (31.6)	14 (16.3)	17 (8.1)	66 (16.3)
3 - 8	46 (41.4)	71 (82.5)	182 (87.1)	299 (73.6)
Total	111 (100)	86 (100)	209 (100)	406 (100)

Table 2: Strain distribution according to resistance profile.

Antimicrobial	Range (µg/mL)	Susceptible N (%)	Intermediary N (%)	Resistant N (%)
Ceftiofur	0,25-8	384 (94.6)	17 (4.2)	5 (1.2)
Amox/Clav	1/0,5-32/64	276 (68.0)	107 (26.4)	23 (5.7)
Ampicillin	1-64	128 (31.5)	-	278 (68.5)
Meropenem	0,25-8	406 (100)	-	-
Fosfomycin	8-512	400 (98.5)	5 (1.2)	1 (0.2)
Oxytetracycline	2-32	101 (24.9)	47 (11.6)	258 (63.5)
Chloramphenicol	4-64	149 (36.7)	48 (11.8)	209 (51.5)
Florfenicol	0,5-8	99 (24.4)	104 (25.6)	203 (50.0)
Nalidixic acid	8-128	114 (28.1)	-	292 (71.9)
Ciprofloxacin	0,06-8	106 (26.1)	69 (17.0)	231 (56.9)
Marbofloxacin	0,06-8	303 (74.6)	14 (3.4)	89 (21.9)
Gentamycin	0,5-32	225 (55.4)	5 (1.2)	176 (43.3)
Neomycin	4-16	363 (89.4)	-	43 (10.6)
Azithromycin	4-64	241 (59.4)	-	165 (40.6)
Colistin	1-16	368 (90.6)	-	38 (9.4)
Sulfamethoxazole	256-1024	135 (33.3)	-	271 (66.7)
Trim-Sulf	2/18-4/76	256 (63.1)	-	150 (36.9)

Amox/Clav – amoxicillin / clavulanic acid; Trim-Sulf – Trimethoprim - sulfamethoxazole.

Discussion and Conclusions

It was observed a significant increase in antimicrobial resistance and multidrug-resistant strains between 2004 and 2018. Increased isolation of multidrug-resistant *S. enterica* is considered alarming and it is a major public health issue. Many factors contribute to the emergence of multidrug-resistant bacteria; however, the selective pressure imposed by the indiscriminate amount and diversity of antimicrobial agents used in animal production is considered one of the main cause of the problem and demands attention from the veterinary community. It is necessary to emphasize in the production sector that prophylaxis, proper animal management and hygiene are basic foundations for diseases prevention that effectively contribute to the increase of productivity, and that can also help to reduce bacterial resistance.

Acknowledgments

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Prevalence of *Lawsonia intracellularis* in Europe

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Introduction

The bacterium *Lawsonia intracellularis* is widespread in all pig populations worldwide (1–4). Studies of individual European countries also suggest a wide distribution (5,6) but are difficult to compare with each other, due to different investigation methods and sampled age categories. This study provides an overview of the prevalence of *Lawsonia intracellularis* in different European countries all measured by the same diagnostic methodology and same age categories. Thereby country specific differences were shown.

Materials and Methods

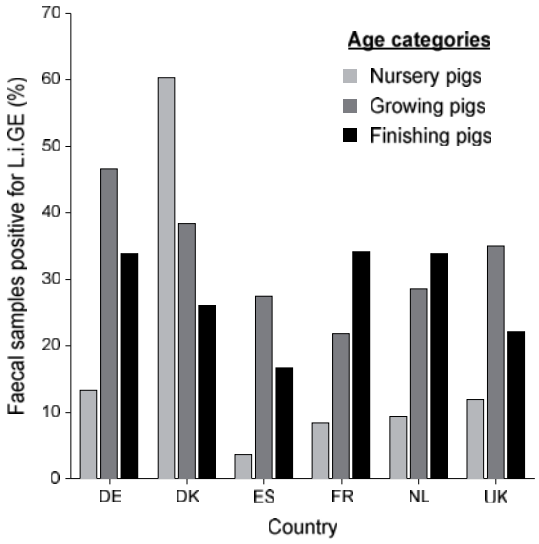
From October 2017 until November 2018, faecal samples were taken from nursery, growing and finishing pigs in six European countries. 24 herds in each, Germany, Denmark, Spain, France, the Netherlands and the United Kingdom participated and overall 6450 faecal samples could be analyzed by quantitative real time polymerase chain reaction (qPCR). Inclusion criteria for the study contained a production system of farrow-to-finish farm or a nursery-/ fattening-farm, receiving all animals from one single origin. In addition, there had to be at least one outbreak of diarrhea in the preceding 12 months, before the date of the examination. Antimicrobial treatment was not accepted within four weeks prior to sampling.

Results

In 90.3 % of all 144 herds sampled, at least one faecal samples was positive by qPCR. Amongst the animals, 26.2 % were tested positive. Thereby samples from Denmark were more often positive than samples from Spain or the United Kingdom. Furthermore, nursery pigs from Denmark were more often positive and shed significantly more genome equivalents than nursery pigs in all other countries (Graph 1). In addition, the concentration of genome equivalents measured by qPCR in Danish herds, was significantly higher compared to the others.

Conclusions and Discussion

A widespread of *Lawsonia intracellularis* in European pig herds was confirmed. The proportion of animals and age categories affected varies from country to country. Country specific differences were found with Denmark in particular diagnosing more *Lawsonia intracellularis* then the other countries tested. Shedding was, in contrast to the other countries, already strongly seen among Danish nursery pigs (Graph 1).



Graph 1. Percentage of faecal samples positive for *Lawsonia intracellularis* per age category and country. DE= Germany, DK=Denmark, ES=Spain, FR= France, NL= Netherlands, UK= United Kingdom; L.i.= *Lawsonia intracellularis*, GE= Genome Equivalents

It appears that mostly these young affected animals have excreted high levels up to 10^6 genome equivalents/ µl. Such high values were much less frequently or not at all achieved in the other countries. It is speculative whether this is due to the fact that animals in other countries become diseased later in age and the immune system then reacts more effective to the infection, or shedding is higher due to another unknown reason. Herd data collected in this study are currently being processed to get more information about possible reasons and risk factors for the differences found between the countries.

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Seasonality of transport mortality and slaughter condemnation related to *Actinobacillus pleuropenumoniae*-like lesions in a slaughterhouse in Sao Paulo

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Introduction

Porcine pleuropneumonia related to *Actinobacillus pleuropneumoniae* (App) is one of the major diseases resulting in increased mortality, medications cost and feed conversion rate and lower growth and weight at slaughter. Moreover is one of the causes related with slaughter partial or total condemnations because the pleurisy lesions producing damage of costal pleura.

The objective of this work was to assess the mortality during transport and the condemnations by App-like lesions.

Materials and Methods

The animals studied were from a 5,200 Topigs-Norvins sows farm located in São Paulo state (Brazil). The company has its own slaughterhouse in the same state and kill all animals from this farm. The productive system was two-sites production with farrowing and nursery un the same location, and finishers in a separate location. The pigs were Apx IV positive by serology and the farm showed App related symptoms during the studied period.

Samples were tooked at slaughter and in farm to confirm the presence and the serotype of App present in the farm. The animals dying during transport and the total or partial condemnations related to App-like pleurisy were recorded during the period form 2017 January to 2019 August. Finally, the results from 287.782 pigs were analyzed. The seasonality index was calculated assuming than 100 is the normality, values higher are related with high prevalence and values under 100 are related to decreased prevalence.

Results

The total average±SEM for pleurisy-related condemnations was 1.66±0.26%, and for mortality during transport was 0.29±0.04%. The yearly record for 2017, 2018 and 2019 for both parameters were 0.834±0.236, 2.577± and 1.52±0.49 for condemnation and 0.150±0.02. 0.304±0.05 and 0.480±0.09 for mortality during transport.

The seasonal indexes are shown in table 1. The higher probabilities of condemnation were recorded in May and the period November-January. The mortality during transport showed higher probability during January-March and September.

A positive significant correlation between mortality and condemnations was found including all data (r=0.376, p=0.032) or removing outlayers data (r=0.411, p=0.011).

Table 1. Seasonal index for mortality during transport and condemnations by pleurisy at slaughter.

Month	Seasonal index	
	Mortality during transport	Condemnations by pleurisy
January	**101.23**	**116.75**
February	**104.32**	96.97
March	**106.84**	50.92
April	98.27	99.82
May	96.06	**108.17**
June	99.99	95.00
July	93.10	63.60
August	99.81	79.54
September	**105.45**	52.22
October	99.20	46.30
November	97.21	**145.15**
December	98.64	**124.20**

Conclusions and Discussion

The frequency of adhesive pleurisy vay very widely among records an countries, being recorded 2.7-12% in Netherlands[1,2] , 14-25% in Denmark[3,4] or 26% in Spain[5]. Thus, in Brazil had been recorded prevalences between 0.005-1.45% in two different studies[6,7].The prevalence found in our study is un accordance to data from Bueno et al[7], and lower than records for Europe. The absence of a major virus such as PRRSv can explain the difference.

The mortality in transport is related to high temperatures, and the higher seasonal index are coincidetn with summer season in southern hemisphere. The correlation between condemnations and transport mortality could be eplained by a higher mortality of animals with lesions related with App.

In conclusion, the mortality during transport and pleurisy-related condemnations showed a clear seasonality and were correlated probably because the increased number of lesions during some months fo the year in Saõ Paulo.

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Reproductive and nursery performances in Brazilian farms with and without symptoms related to *Actinobacillus pleuropneumoniae*

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Introduction

There is no wide information about the influence of *A. pleuropneumoniae* (App) on reproductive performances, although it is well known that the health status is absolutely related to these parameters. In this study, we have recorded the reproductive performances in farm with and without symptoms related to *A. pleuropneumoniae* in nursery and finishing.

Materials and Methods

A total of 14 farms, ApxIV positive, were included in this study, classified depending on the appearance of symptoms related to App during nursery or finishing. The sample included three different genetic lines. Moreover, a diagnosis of the bacteria was made on the basis of isolation, and identification by Gram staining, biochemistry panels and Exopol EXOone App Basic Kit (Exopol, Spain). The farm were then qualified as *presence of symptoms* (PS) or *absent of symptoms* (AS) if there were clinical signs related to the disease, and a isolation or identification by PCR of the bacteria.

The average yearly data during the period 2017-2019 were included in a database, analyzing number of sows, Fertility, Sows in heat into 7 days post-weaning (SIH7) Total born piglets per litter (TBP), Alive born piglets by litter (ABP), Weaned piglets by litter (WP), Sow's mortality (SM), Total weaned/sow/year (TWSY) weight at weaning (WW) and Age at weaning (AW). During nursery, were recorded Mortality (NM), average daily gain (ADG), Feed conversion rate (FCR) and weight at tranfer to finishing (WTF), and age at tranfer (AT).

The average cost of piglets was calculated on the basis of the reproductive performances. a Student's t for independent samples was performed.

Results

Five farms were classified as PS (35.72%) and ten as AS (64.28%). There was no difference in the average census of the farms (2,358±355 vs. 2,017±473 for AS and PS farms, respectively) The reproductive performances appear in the Table 1, and the performances for nursery in Table 2.

There were no differences comparing the genetic lines into PS and AS groups, discarding the influence of this factor on results.

On the basis of the economic results, a difference of 2.28€ per piglet was calculated, comparing the PS with the AS group.

Table 1. Reproductive performances for AS and PS groups.

Parameter	AS	PS	P
Fertility	90.27±0.49	88.37±0.65	0.028
SIH7	93.31±0.86	87.11±2.3	0.004
TBP	14.79±0.12	14.00±0.14	<0.001
ABP	13.52±0.10	12.5±0.12	<0.001
WP	12.18±0.12	11.30±0.15	<0.001
SM	9.91±0.65	7.97±1.17	NS
TWSY	30.05±0.29	27.20±0.34	<0.001
WW	6.39±0.315	5.76±0.27	<0.001
AW	23.64±0.27	22.22±.32	0.002

The only parameter showing no difference was sows mortality, even higher in AS group.

Table 2. Performances in nursery for AS and PS groups.

Parameter	AS	PS	P
NM	1.64±0.13	2.00±0.18	NS
ADG	402.7±5.59	371.18±14	0.017
FCR	1.516±0.02	1.664±0.05	0.002
WTF	23.36±0.44	21.13±1.1	0.028
AT	64.26±0.54	63.46±1.83	NS

There were differences for ADG (p=0.035) and ML (p=0.004) comparing genetics lines for the PS groups, but no for any parameters into AS group.

Conclusions and Discussion

There is a lack of information about the influence of App on reproductive performances, although it's well known the influence of other *Actinobacillus*. Thus, *A. suis* can produce 7% of later abortions in sows and gilts as demonstrated in Croatia[1]. Regarding App has been recorded an increase in mortality of piglets during suckling, arising up to 10.9%[2], which, unfortunately can be considered as normal nowadays.

The lower reproductive performance for PS groups could be an indicator of poorer heatlh status compared to AS group, being the differences due to more factors than App alone.

The difference of performances during nursery is especially important on economically critical factors such as ADG or FCR. This is the explanation of the difference in piglets cost. It is known that even a subclinical infection by App can results in poor performances and increased costs.

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Antimicrobials susceptibility in Actinobacillus pleuropneumoniae strains isolated in 20 Brazilian farms

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Introduction

The resistances to antimicrobials are nowadays a major concernment in livestock industry, and especially in swine production. Not only the implications in public health but also the lack of results in prevention and treatment of pig's diseases pushed the producers and veterinarians to consider more and more the needs of a responsible and rational use of antimicrobials.

Here we described the resistances found in the *A. pleuropneumoniae* strains isolated from 20 Brazilian farms during 2016-2019 period.

Materials and Methods

During the formerly referred time period, samples obtained in farm on slaughter were sent to CEPPA laboratory (Paulínia, São Paulo) from 20 farms with or without Pleuropneumonia symptoms. The samples were lung pieces. The samples were seeded on selective media; App was identified by Gram staining and biochemistry panels. The specific serotype was investigated by means of Exopol EXOone Basic Commercial Kit (Exopol, Spain), able to identify individually serotypes for 1 to 16, except for 9-11 that are undiferenciable.

The isolated strains were reseeded and an antibiogram using the Kirby-Bauer method by means of commercial disc was performed. The antimicrobials tested were Amoxicillin, Ceftiofur, Doxiciclyne, Enrofloxacin, Florfenicol, Gamithromycin, Lincomycin+Spectinomyicin, Oxytetracicline, Penicillin, Tetracycline, Tilmicosin and Tulathromycin.

Based on the width of inhibition halo, the strains were classified as susceptible, resistant or intermediate. The frequency of simultaneous resistence profiles was analyzed by means of squared Chi and adjusted residuals analysis.

Results

Finally, 38 strains were isolated, always from farms showing pleuropnuemonia symptoms. In some cases, two different strains were identified in the same sample. Interestingly, the strain 5 did not show any resistance. Individually analyzed, 91.76% of strains were resistant to Oxytetracicline and Penicillin, 39.13% to Amoxicillin, 36.36 to Doxiciclyne, 32% to Enrofloxacin and 28.57% to Tetracycline. It was found up to eleven different patterns of simultaneous resistances. The frequency and serotypes appear in the Table 1.

Up to four simultaneous resistances (AmoDOxPenTet) were found in a serotype 7 strain.

The most frequent was AmoxPen, followed by AmoxDoxPen and OxiPen. There was a significant higher frequency ($p<0.001$) for the pattern AmoxPen for serotype 7, OxiTet for serotype 15, AmoxDoxPen and AmoDoxTet for the combination of 4 and 7, and DoxOxiPen for the combination 7 and 12.

Table 1. Frequency for simultaneous resistances patterns depending on the serotype

Pattern	%	Serotypes	Adjusted residuals
Pen	2.7	7	
AmoxPen	24.32	7	3.1*
AmoxDox	5.41	4 and 7	
DoxPen	2.7	7	
OxiPen	13.51	7	
OxiTet	5.41	15	5.8*
AmoxDoxPen	16.22	4 and 7	3.9*
AmoDoxTet	8.11	4 and 7	2.6*
Amo PenTet	5.41	7	
DoxOxiPen	5.41	7 and 12	5.8*
AmoDoxPen Tet	2.7	7	

* $p<0.001$

Conclusions and Discussion

The highest frequencies of resistance were found for the most commonly used antimicrobials and, interestingly, all strains were susceptible to the last generation antibiotics, as previously was recorded in other studies[1,2]. The resistances to Amoxicillin or Doxycycline are in accordance with other previously recorded in some countries. The fact that the most frequent combined patter was AmoxPen is due both antibiotics share the resistance mechanism. The appearance of nine patterns with simultaneous resistance to β-lactamics and tetracylcines could be the result of combination of different genes such as bla_{ROB-1} y *tet*B as previously described for App[3,4], but more research in needed for this aspect.

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Serotypes of *Actinobacillus pleuropenumoniae* identified in 20 Brazilians farms during 2016-2019

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Introduction

Porcine pleuropneumonia related to *Actinobacillus pleuropneumoniae* (App) is one of the major diseases resulting in increased mortality, medications cost and feed conversion rate and lower growth and weight at slaughter. Up today, 16 serotypes of the pathogen have been identified. And currently, there are the adequate tools to identify, specifically, each one of them.

The objective of this work was to assess the presence of App and to identify the serotypes present in 20 farms in Brazil.

Materials and Methods

Twenty (20) farms were selected in Minas Gerais (n= 4), Mato Grosso (n=3), Santa Catarina (n=1), Paraná (n=7) and São Paulo (n=5) states in Brazil, totalyzing 40,880 sows. All the farms were ApxIV positive by serology.

The farms were classified as presence of absence of pleuropneumonia-like symptoms.

Samples were taken from lungs or tonsils at farm or at slaughter, fomr app-like lesions. The presence of App was assessed by means of Exopol EXOone App Basic Kit (Exopol, Spain), and the specific serotype was investigated by means of Exopol EXOone Basic Commercial Kit (Exopol, Spain), able to identify individually serotypes for 1 to 16, except for 9-11 that are undiferenciable.

One of the farms (Farm G); sited in São Paulo state (5,200 sows, two sites production system) was selected to be sampled onn a continuos basis durin 2017-2019. Thos farm was selected because the company have their own slaughterhouse, allowing a more easy inspecion and sampling.

Results

A total of 8 farms (40%) showed App-related symptoms; 3 in Minas Gerais (n= 75%), 2 in Mato Grosso (n=66.7%), 4 in Saõ Paulo (80%), and Santa Catarina (n=1), Paraná (n=7) and none in Paraná and Santa Catarina.

App was identified in all the farms showing symptoms and none of the farms without symptoms.

The serotypes found were 4, 5, 7, 8, 12 and 15 with a frequency of 4, 4, 64, 4, 12 and 12%, respectively.

In Farm G, were done 18 isolations and identifications; 5 in 2017, 9 in 2018 and 5 in 2019. In 11 identifications was found the serotype 7 (n=61.1%) alone, in 5 was identified simultaneously serotypes 4 and 7 (27.77%), in 1 were identified serotypes 7 and 12 (5.55%) and in 1 were identified simultaneously serotypes 7, 8 and 15 (5.55%). All the isolations during 2017 and the first half of 2018 were serotype 7 alone, All the findings during 2019 were serotypes 4 and 7 together.

Conclusions and Discussion

The frequency of findings for different serotypes vary among countries. Our data indicates that serotype 7 is the most prevalent in the studied farm, found in 100% of the isolations. The predominance of serotype 7 has been recorded in Spain[1] and Chile[2]. Generally, a low prevalence for serotypes 4, 5 and 12 is recorded, except for 5 in Corea[3], Filipinas[4] and Canadá[5], or 12 in Australia[6]. In Brazil in 2007, the most frequently isolated serotypes were 3, 5, 6 and 7; with a frequency of 7-13%[7] for this last serotype, very lower than our findings.

The posibility of sequential infections by different serotypes has been previously recorded.

However, there is no information about the presence of serotype 15 in Brazil. In fact, there is no a wide record of this serotype. The highest prevalenve has been recorded in Australia[8] wit 33% of isolations. Up to our knowledge is the first record of serotype 15 in Brazil.

We can conclude that the prevalence of App is dynamic, and a farm can be sequentially infected by different serotypes. In this case, a farm infected with serotype 7, was subsequently infected by 4, 8, 12 and 15. The presence of 12, 8 and 15 could be related with an increase of lesions at slaughter (data not shown). The serotype 15 is described for the first time in Brazil.

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Pleurisy evaluation in slaughtered pigs and it´s effect on the average daily weight gain in batches with/without an *Actinobacillus pleuropneumoniae* vaccines

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Introduction

Pleurisy caused by *Actinobacillus pleuropneumoniae* (App) is a common disease in Mexico. Economic looses associated are referred to the productive parameters in grower and finishing stages. Losinger W. study indicate looses by $32M UsDlls due to the App infection (1), Mores *et al* conclude in an 8.0M Usdlls looses by low carcass weights and slaughter confiscations by App pleurisy The objective of this study was to evaluate pleurisy in slaughter and it´s effect on the average daily weight gain (birth to sales) from different pig batches with the use or not of an *Actinobacillus pleuropneumoniae* vaccines.

Materials and Methods

1370 lungs from 29 production batches were evaluated in slaughterhouse. For each batch we obtain total weight and average age in days. ADWG (Average Daily Weight Gain) from birth to market were calculated in each batch. During the slaughter process all lungs were analized by the SPES (Slaughterhouse Pleuritis Evaluation System) method reported by Dottori et al. (3) .Pleurisy lesion score from 0 to 4 and frequency were used to obtain the App Index in each batch. From the total analyzed batches (29) 7 didn't use any App vaccine, these farms never experience any signs or mortality associated to App infection, total lungs in this group were 279. From the rest 22 batches with 1091 lungs the farms use different App vaccines to control clinical signs and mortality. App Index and ADWG were analized with an ANOVA using a Tuckey test. by SPSS V15.0 software.

Results

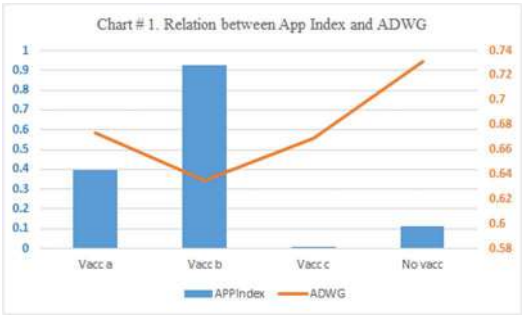
Table # 1 show the App Index and ADWG by each vaccine and no vaccine lungs and batches, statistics differences were only detected in vaccine a and b vs no vaccine batches. For ADWG there were no differences ($p \leq 0.05$)

The graph # 1 show relation between APP-Index and ADWG in all groups.

Table # 1. App Index values and ADWG Birth-Slaughterhouse in days

Vaccine ID	Lungs n	Batches n	App Index Median (Std error)	ADWG Median (Std error)
a	821	17	0.397^{a} (± 0.089)	0.674^{a} (± 0.011)
b	120	3	0.924^{a} (± 0.055)	0.635^{a} (± 0.045)
c	150	2	0.0075^{ab} (± 0.0069)	0.669^{a} (± 0.014)
No Vaccine	279	7	0.114^{b} (± 0.0078)	0.731^{a} (± 0.26)

Different superscripts in the same column indicate statistic differences ($p \leq 0.05$)



Conclusions and Discussion.

App infection caused chronic lung lesions detected in slaughterhouse, early infections (less than 15 weeks age) can affect the growth performance by reducing ADWG, this was demonstrated in this study if we compare farms without App infection vs affected ones, difference in this parameter was 72.0g even there were no statistic differences. For the three groups with vaccine use there is no a clear relation between both parameters ($R^2 = 0.002$) maybe due to the difference in the App Index however the highest one (0.924) in vaccine b group showed the lowest ADWG (0.635). Lung lesions, clinical signs and mortality by an App infection contribute in the reduction of productive parameters, the use of vaccine prevent it´s effects in mortality and clinical signs.

Acknowledgments

Sonora state farms that allowed the slaughter analysis.

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Pharmacokinetics of Apravet® 100 g/kg premix (apramycin sulphate) following single oral administration in pigs

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Introduction

Apramycin (APM) is an aminoglycoside antibiotic for veterinary use which acts mostly against Gram-negative bacteria.[3] The aim of the study was to investigate the pharmacokinetics (PK) of APM applied orally to pigs as Apravet® 100 g/kg premix for medicated feeding stuff for pigs (Huvepharma® NV).

Materials and Methods

Eight healthy pigs, equal number of male and female, weighing 11.5-13.5 kg without a history of aminoglycoside administration were enrolled in the study. The animals received by intubation a single p.o. dose of APM (as sulphate) 40 mg/kg b.w. (Apravet® 100 g/kg premix for medicated feeding stuff for pigs).[1] Heparinized blood samples were taken at predetermined intervals up to 24th h. APM plasma concentrations were determined by a validated HPLC method. Main PK parameters were calculated by non-compartmental PK model.

Results

Plasma concentration vs. time data fit best by applying the non-compartmental model. The highest plasma concentration (C_{max} = 2.229±0.08 µg/mL) was achieved 1.5 h post administration. Thereafter, plasma levels declined remaining higher than 0.657±0.02 µg/mL at 12 h post administration (Fig. 1). The half-life ($t_{1/2\beta'}$) was 10.22±0.46 h, $AUC_{0\text{-}tlast}$ and $AUC_{0\text{-}\infty}$ were 14.38±0.58 µg.h/mL and 24.01±0.74 µg.h/mL, respectively (Table 1).

Table 1. PK parameters of APM in pigs following single oral administration of Apravet® 100 g/kg premix at a dose of 40 mg/kg b.w. APM (mean±SEM)

Parameters, units	APM, 40 mg/kg BW
β' (h^{-1})	0.0687 ± 0.003
$t_{1/2\beta'}$ (h)	10.22 ± 0.46;10.15*
C_{max} (µg/mL)	2.229 ± 0.08
T_{max} (h)	1.5 ± 0.0
AUC_{tlast} (µg.h/mL)	14.38 ± 0.58
$AUC_{0\text{-}\infty}$ (µg.h/mL)	24.01 ± 0.74
MRT (h)	13.70 ± 0.53
t_{last} (h)	12.0

• – harmonic mean time

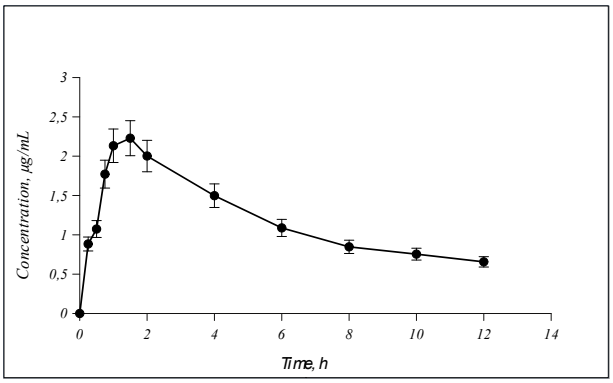

Figure 1. Mean serum concentrations of APM in pigs following single oral administration of Apravet® 100 g/kg premix at a dose of APM 40 mg/kg b.w.

Conclusions and Discussion

The PK parameters of APM determined in the present study are considered typical of aminoglycoside antibiotics after oral administration to pigs. Our results correspond with the reports on the PK of APM in pigs and demonstrate that APM is poorly absorbed and tissue distribution is limited after oral administration.[2,3]
Therefore, the results are helpful for understanding the PK characteristics and correct administration of APM to pigs. These data support the clinical use of APM as Apravet® 100 g/kg premix in the control of gastro-intestinal infections.

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Eradication of *Mycoplasma hyopneumoniae* by strategic medication with Vetmulin®

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Introduction

Despite vaccination against *M. hyopneumoniae*, pigs originating from a birth to weaning herd of 700 sows were still suffering from chronic coughing, poor growth and increased mortality caused by Enzootic Pneumonia. The weaners (27 days old) were moved weekly to a compartmentalised nursery site and finally at 11 weeks of age transferred to all-in all-out finishing stables, all at different sites. A farm-specific eradication programme without farrowing stop based on strategic medication with tiamulin (Vetmulin® 100g/kg microgranulated premix - Huvepharma®) was initiated to improve the general health status. A strict hygiene and disinfection protocol was installed. Only gilts originating from negative herds were introduced afterwards.

Materials and Methods

Animals refusing to eat before and during the treatment period were removed. The remaining sows and gilts were treated via feed with a daily dose of 10 mg tiamulin hydrogen fumarate/ kg bodyweight for 2 weeks. From 1 week before till the end of the treatment period, the suckling piglets received a dose of tulathromycin (2.5 mg/kg IM) every 5 days. Piglets born thereafter were considered to be *M. hyopneumoniae* free and were no longer vaccinated against *M. hyopneumoniae*. These pigs were transferred to completely depopulated, clean and disinfected facilities for weaners and fatteners. Mycoplasma monitoring was initiated after treatment cessation to evaluate the eradication success. As a first step, the oldest weaners (11 weeks old) were serologically tested (n=25, ELISA) and PCR analysis was performed on oral fluids (n=9) and nasal swabs (n=3 pools of 5) of sows, gilts and weaners 14 weeks after the end of the strategic medication programme. A second monitoring was performed in 4 different locations 23 weeks after the end of the treatment. At that moment blood samples were taken of sentinel gilts (n=20) in the sow farm and weaners at an age of 11 weeks (n=20) in the nurseries. In addition, 2 fattening units at different locations, housing 17 and 16 weeks old pigs were tested by serology (n=20 each) and PCR on oral fluids (n=3 each). Finally, 10 sentinel gilts and 20 heavy pigs in both fattening units (24 and 23 weeks old) were serologically tested 30 weeks after the cessation of the treatment. Furthermore, the impact on the zootechnical performance was investigated.

Results

All ELISA serology (n= 155) and PCR tests (n= 18) performed after the strategic medication programme were negative for *M. hyopneumoniae.* Clinical signs of enzootic pneumonia were no longer observed in the 4 herds. A clear improvement of the general health status and technical performance of both weaners and finishers born after the eradication was noted.

Table 1. Impact of the *M. hyopneumoniae* eradication programme on technical performance (data from the last 16 weeks before and first 13-16 weeks after treatment).

		Pre	Post	Diff.
Farrowing units				
-Mortality	%	13.6	12.3	-1.3
-Weaning weight	kg	6.53	7.23	+0.7
Nurseries				
-Exit weight	kg	28.93	31.80	+2.87
-Daily weight gain	g/d	415	468	+53
-Mortality	%	3.14	1.98	-1.16

For preparation of this paper, final technical results of the first batch of *M. hyopneumoniae* negative fattening pigs were not yet available. Nevertheless, a clear reduction of the respiratory symptoms and improved general health status was already observed in these pigs.

Conclusions and Discussion

M. hyopneumoniae can successfully be eradicated by a strategic medication with tiamulin (Vetmulin®) in combination with biosecurity measurements. This programme doesn't require any depopulation of the sows or farrowing stop and ensures optimal animal welfare, improved health status and productivity.

Evaluation of three commercial ELISAs for detection of antibodies against *Mycoplasma hyopneumoniae* in serum samples

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Introduction

Serological tests are commonly used to monitor the health status of pig herds. ELISA is a rapid, inexpensive and easily automated diagnostic method for *Mycoplasma hyopneumoniae* (*M. hyo*) that provides useful information on the presence of antibodies and seroconversion. However, differences in sensitivity and specificity of *M. hyo* ELISA kits have been reported (1).

This study compares the performance of three commercial *M. hyo* antibody ELISAs using serum samples from pigs of known status.

Materials and Methods

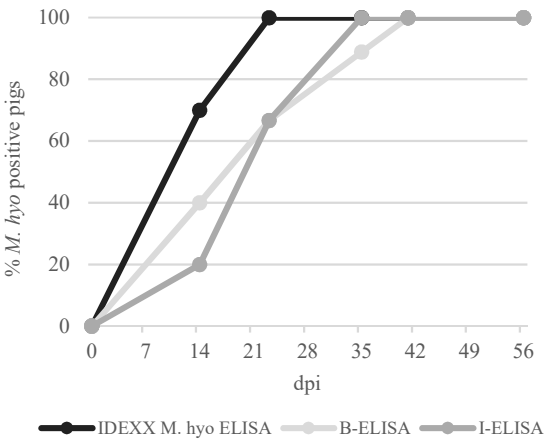
A total 1,216 serum samples were used to evaluate the sensitivity and specificity of three commercial *M. hyo* antibody ELISAs. For the specificity study, 500 serum samples from *M. hyo* naïve pigs were included. For the sensitivity study, 671 serum samples from *M. hyo* positive pigs were used. The presence of *M. hyo* was confirmed on tracheal swabs at the pen level before sampling. In addition, serum samples (*n*=45) from ten pigs experimentally infected with *M. hyo* were collected at 14-, 23-, 35-, 41- and 56-days post infection (dpi) and used for the onset of detection study. The experimental design of the infection and transmission study has been described previously (2). Serum samples were tested with (i) IDEXX *M. hyo* Ab Test [IDEXX Laboratories, Inc., Westbrook ME, USA] (IDEXX *M. hyo* ELISA), (ii) another commercial *M. hyo* antibody indirect ELISA (I-ELISA) and (iii) a blocking *M. hyo* ELISA (B-ELISA) according to the manufacturer's recommendations. For the sensitivity calculations, positive and suspect results were grouped together.

Results and Discussion

Both indirect ELISAs, IDEXX *M. hyo* ELISA and I-ELISA, correctly identified known negative serum samples (Specificity = 100%). The B-ELISA failed to correctly identify four negative serum samples (Specificity = 99.2%), with three positive and one suspect results.

Following the experimental infection, the IDEXX *M. hyo* ELISA detected 70% of positive pigs at 14 dpi and 100% at 23 dpi. However, the I-ELISA was only able to detect 20% and 66.7% of positive animals and the B-ELISA 40% and 66.7% at 14 and 23 dpi, respectively (Figure 1).

Figure 1. Onset of detection of three *M. hyo* antibody ELISAs.



The better onset of detection showed by the IDEXX *M. hyo* ELISA, with a higher percentage of positive pigs detected as early as 14 dpi, supports its use for early diagnosis of *M. hyo* infection and detection of index cases compared to the other ELISA kits evaluated.

The sensitivity of the IDEXX *M. hyo* ELISA was higher than the other indirect ELISA (I-ELISA) included in this study, detecting 58 positive samples more (8.1%).

Conclusions

In summary, the IDEXX *M. hyo* Ab Test showed 100% specificity, better than a blocking *M. hyo* ELISA, higher sensitivity than another *M. hyo* indirect ELISA and better onset of detection than the indirect and blocking ELISAs evaluated in this study.

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Elimination of *Mycoplasma hyopneumoniae* in a farrow-wean facility using herd-specific homogenate

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Introduction

A high-health 2500-sow farrow-wean facility became infected with *Mycoplasma hyopneumoniae* (Mhp). To eliminate the disease, an aggressive program of exposure, cleaning, disinfection, and herd closure (no introductions to the defined population) was utilized. Aerosol exposure to an autogenous Mhp inoculum was utilized to expedite uniform Mhp colonization of replacement gilts. This overview outlines the key components of a successful Mhp elimination.

Materials and Methods

A high-health 2500-sow farrow-wean population, confirmed to be Mhp-negative since stocking was exposed to Mhp by introduction of Mhp-infected replacement gilts in December 2017. Mhp infection was confirmed the first week of January 2018 (1wk18). Exposure of the resident population began with direct contact between clinical and non-clinical animals. Heat-check boars received particular attention and were used to spread Mhp through the gestating herd.

A homogenous Mhp inoculum was produced from lung samples of two clinical animals 4wk18. Based on gross lesions, only clinically affected sections of each lung were used. The lung from each donor was homogenized using a commercial food blender with culture medium as an extender. Inoculum was stored in a freezer until the replacement gilt population could be identified and assembled. One group of replacement gilts was exposed to the inoculum diluted with Friis medium (Technova) 7wk18. The remainder of the replacement gilts were similarly exposed 8wk18. The inoculum was aerosolized using a commercial fogger (Curtis Dyna-Fog) in a small confined space (enclosed livestock trailer). Conditions in the trailer were intentionally warm to encourage deep breathing in the hope of deeper exposure and colonization. Strategic PCR testing of tracheal samples combined with serum antibody tests (ELISA, IDEXX) across the replacement gilt population and resident sow populations were used to establish a Day0, the date assumed all animals were effectively Mhp colonized. Based on these results, 14wk18 was determined to be the start of the herd closure period.

The entire sow herd was vaccinated with commercial Mhp vaccine (Respisure, Zoetis) 22wk18 and 25wk18. The sow herd (both gestation and lactation) received feed-grade tilmicosin (Pulmotil, Elanco) during 46-50wk18. Piglets born 46-50wk18 received a weight-appropriate dose of tulathromycin (Draxxin, Zoetis) at 1 and 10 days of age. During the treatment period, maximum weaning age was 18 days.

Using a minimum of 240 days for herd closure, piglets suspected to be Mhp-negative were born 50wk18 and weaned into all-in/all-out wean-to-finish facilities 2wk19. Confirmed Mhp-negative replacement gilts entered the resident population 3wk19.

Results

Aggressive PCR testing on tracheal samples confirmed the pigs remained free of Mhp through the growing period. The absence of clinical respiratory signs supported the Mhp-negative status. Select individuals from the first suspected Mhp-negative pigs were sampled at 22 weeks of age 20wk19 and confirmed Mhp-negative.

Mhp-negative replacement gilts exposed to the resident population (sentinels) were sampled over time and remained Mhp-negative based on PCR testing of tracheal samples, ELISA (IDEXX) testing of serum and absence of clinical signs.

The resident sow herd and downstream flow remain Mhp-negative at the time of publication, more than one year after the weaning of the first Mhp-negative piglets.

Conclusions and Discussion

Successful inoculation under these conditions offers a useful tool to achieve uniform and predictable exposure leading to colonization of Mhp as the first step of Mhp elimination. With the exposure processes described, it took only 7 weeks from inoculation to complete colonization of the gilt population. Standardized effective exposure early in the process and confirmation of colonization aid in establishing an appropriate period for herd closure. Strict herd closure during the period of exposure, colonization, building of immunity and clearance of the pathogen is required for the overall success. Based on experience, herd closure >240 days is necessary for predictable success. Success or failure of the elimination process is determined by testing both downstream flow and replacement gilts entering the resident population.

Acknowledgments

The authors acknowledge the conviction of the owners and the dedication and attention to detail of the committed farm staff, required to make this exercise a success.

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Use of 5-step process to control *M. hyopneumoniae* in a Spanish farm

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Introduction

Mycoplasma hyopneumoniae (M.hyo) is the primary pathogen of enzootic pneumonia, a chronic respiratory disease in pigs. Infections with *M. hyo* are highly prevalent in almost all swine producing areas, and they cause significant economic losses due to increased medication use and decreased performance of the pigs. Moreover, *M. hyo* is also considered to be one of the primary agents involved in the porcine respiratory disease complex (PRDC)[1].

In order to control this disease Boehringer Ingelheim Vetmedica, Inc. (BIVI) has developed a systematic platform, known as the M.hyo 5-step *process*. The 5-step *process* consists of 5 basic steps: identify desired goals, determine current status, understand current constraints, develop solution options, implement and monitor the preferred solution. The aim of this study was to implement a control program to obtain a better knowledge of the dynamics of the infection and reduce the M.hyo colonization in piglets.

Materials and Methods

The 5 step process was conducted in a 250 sows 2-sites farrow-grower farm, located in Tarragona (Spain). The sow farm is PRRS, App, and M.hyo positive and piglets get vaccinated against PCV2 and M.hyo with FLEXcombo® (Boehringer Ingelheim Vetmedica GmbH) at 3 weeks of age (woa). Every two weeks 250 pigs at the age of about 10 weeks are transferred to a fattening site (continuous flow). Historically, pigs are tested at 3 and 9 woa with ELISA kits (IDEXX) for PRRS, App and influenza.

In May 2018, pigs from 60-80kg onwards started coughing. The parameters registered in that fattening batch were 6% of mortality, 660g average daily gain (ADG) and 2.52 kg feed conversion rate (FCR). At that moment, the 5step process was implemented. The goal was to reduce clinical signs (dry cough) and to improve the productive parameters mortality rate, ADG and FCR.

To determine the current status, 3 samples of oral fluids were taken at 14, 17 and 20 woa and tested by PCR for PRRS, PCV2, Influenza and App. In addition, 10 serum samples were taken at 5 woa and 20 serum samples at the end of the fattening period and tested for PRRS (IDEXX ELISA), App (ApxIV ELISA), PCV2 (Ingenasa IgG/IgM ELISA), M.hyo (IDEXX ELISA) and Influenza (H1N1, H1N2, H3N2 HAH) (Figure1 and Table 1). Results clearly demonstrated that M.hyo control was not sufficient and it was decided to implement several measures to reduce the M.hyo colonization in piglets.

These measures included: double M.hyo vaccination of gilts during the quarantine period, double M.hyo mass vaccination of sows within 4 weeks[2], and peri-partal medication of sows with tiamulin (200ppm) over a period of 5 weeks. In addition, piglets received an extra M.hyo dose at 8 woa for a period of 6 weeks and some batches in the fattening unit were medicated with tilosine.

Figure 1. Positive oral fluids (%) at 14, 17 and 20 weeks of age.

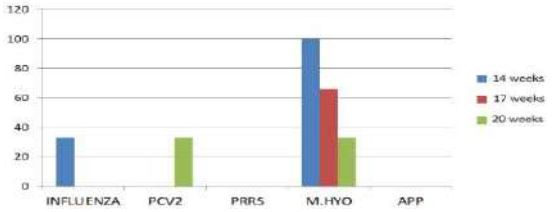

Table 1. Results of serum samples.

	% positive pigs at 5 woa	% positive finishers
ApxIV ELISA	60	0
PCV2 IgG	50	60
PCV2 IgM	20	0
PRRS ELISA 2.0	0	0
M.hyo ELISA	0	80
Influenza HAH	n.a.	40

Results

In March 2019, only 10% of fatteners were positive to M.hyo (IDEXX ELISA), ADG was 743g and FCR and mortality rate were reduced to 2.38 and 1.9%, respectively. As before, pigs remained PRRS negative until the end of the fattening period.

Conclusions and Discussion

In the presented case, the application of the 5step process has helped to improve not only the productive parameters of the fattening pigs, but also the dynamics of M.hyo in the sow farm. A correct gilt acclimation against M.hyo is crucial to reduce the M.hyo colonization in weaned piglets[3]. The 5-step process approach allows a systematic approach to controlling the disease.

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Percentage of positive farms to *Lawsonia intracellularis* in non-vaccinated fattening pigs in Mexico

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Introduction

Swine Proliferative Enteropathy (PPE) is an enteric disease caused by *Lawsonia intracellularis* (*L. intracellularis*) which has a considerable economic impact on swine production. Its subclinical presentation is the most common in animal populations, with sero-prevalences greater than 39% in fattening pigs in México (1), while in Europe prevalence has been reported >90% (2). The objective of this study was to update the prevalence information on *L. intracellularis* in unvaccinated fattening pigs farms in Mexico and to compare differences on prevalence between geographical regions.

Materials and Methods

A cross-sectional study was conducted on 27 farms distributed in 4 different regions in Mexico: northwest, central, west and east. A total of 810 serum samples were collected during October and November 2019. For selection, farms must meet the following criteria: 1) not be vaccinated against *L. intracellularis*, 2) be sampled at 16, 18 and 20 weeks of life (3) do not present clinical signs associated with PPE. A farm would be considered positive when having at least one sample with detection of antibodies to *L. intracellularis*. In total, 30 individual serum samples were taken per farm (divided into 10 serums per age), considering a 95% confidence level and a prevalence of 10%. The diagnostic reagent used for this study was bioScreen Ileitis Antibody ELISA (Svanova, Sweden). A Chi-square test was applied (Minitab 19, USA) for comparing farm-level prevalence between regions.

Results

Table 1 shows that 85% of sampled farms were positive to *L. intracellularis*.

Table 1. Percentage of farms positive to *L. intracellularis*

Status *a L. intracellularis*	%	Farms
Negative	15%	4
Positive	85%	23
Total	100%	27

Figure 1 presents the prevalence at farm level reported by region. The western region had less pevalence when compared with central and north, but not with east. The other three zones have no significant differences among them.

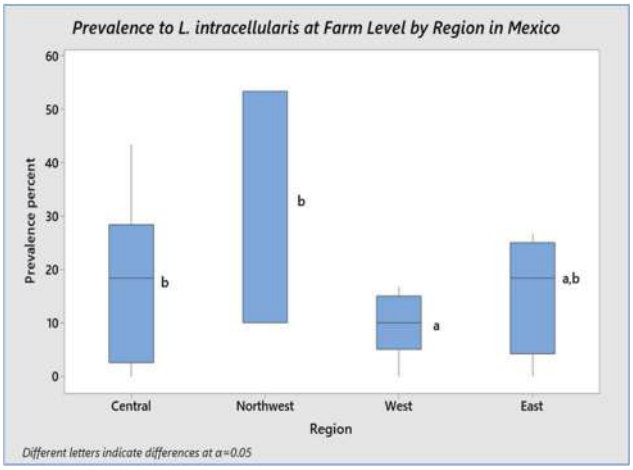

Figure 1. Prevalence to *L.intracellularis* by region.

Conclusion and Discussion

This study updated information on farm prevalence to *L. intracellularis,* observing an increase on farm-level antibody detection when compared to previous data (1,3). One possible explanation for the highest prevalence found in the northwest region is the controlled use of antibiotics in this region for export purposes. Subsequent studies are needed to determine potential correlations between serological results and productive impact at the farm level, leading to the implementation of strategies for the control and prevention of *L. intracellularis* Mexican pig farms.

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Effect of an oral live attenuated vaccine against *Lawsonia intracellularis and Salmonella choleraesuis* on weaned and finished pig performance in a commercial farm in Mexico

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Introduction

Infection by Salmonella is an important risk of public health and the food security of the pork products. Different methods to prevent or reduce the contamination prevalence had been reported. *Lawsonia intracellularis* is a common pathogen of the pig gut and the prevalence ranging 48-100% around the world (1). The infection with *L. intracellularis* is *commonly* present in fattening pigs, which generate economic losses and an increase in the excretion of enteric *Salmonella* (2). The objective of this study was to evaluate the effect of a live attenuated vaccine *L. intracelullaris* and *S. choleraesuis* on some productive traits of pigs from weaning to finishing in a commercial farm.

Materials and Methods

A prospective study where carried out from December 2017 to September 2018 in a commercial farm, located at the western side of Mexico. The farm had 2,000 sows positive to *L. intracellularis* and *S. cholerasuis*. It had one site 1 with exclusive flow from weaning (3 weeks) to finishing. The study was conformed of 46,000 pigs (23,000 pigs per group), measured every week for twenty-six consecutive production weeks (total 52 weeks). One cohort of pigs were vaccinated simultaneously at 7 days of age (2 mL) with two oral attenuated live vaccines: *L. intracellularis*, Enterisol® Ileitis and *S. choleraesuis,* Enterisol® SC-54 (Boehringer Ingelheim). The other cohort was a control group. The vaccination program for both groups was similar (PRRS MLV + PCV2 + *Mycoplasma hyopneumoniae*) 2mL, intramuscularly at 21 days. Mortality, body weight and average daily gain (ADG) data was record from weaning to finishing. The data were analyzed using the SPSS program (SPSS® Statistics), where the means of body weight, ADG, and age at sale were compared using the General Linear Model procedure. Mortality rate was analyzed using Chi square test. In addition, the rate of return on investment and the benefit cost ratio were estimated using the BECAL tool (Boehringer Ingelheim Economic Calculator).

Results

There were significant statistical differences between the two treatments, except for mortality rate (Table 1). A better performance was obtain for body weight at 70 days, age adjust at 100 kg, market body weight and ADG (Table 2), in the pigs vaccinated with oral live attenuated vaccines. These results are similar to those reported by Almond (3). This author observed an improvement in the ADG in pigs that were vaccinated with oral live attenuated vaccines (*L. intracellularis/S. choleraesuis*). Both the return on investment and the benefit cost ratio (4.9 to 1 and 5.9 to 1, respectively) improved when the oral live attenuated vaccines against *L. intracellularis* and *S. cholerasuis* were used, Peiponen (4) reported similar results.

Table 1. Comparison of the mortality rate (%) in pigs under vaccination schemes with oral live attenuated vaccines (*S. choleraesuis / L. intracellularis*)

	Group		
Phase	**Unvaccinated**	**(Sc/Li) Vaccinated**	**Difference**
Nursery	3.52	3.44	-2.3
Finisher	4.80	3.64	-24.0
Wean to Finish	8.13	6.91	-15.0

Sc = *Salmonella choleraesuis* ; Li = *Lawsonia intracellularis*

Table 2. Comparison of productive traits in pigs under two vaccination schemes with oral live attenuated vaccines (*S. choleraesuis / L. intracellularis*)

	Group		
Traits	**Unvaccinated**	**(Sc/Li) Vaccinated**	***P*-Value**
Number of pigs	23,000	23,000	
Initial body weight, kg	5.9 [a]	6.2 [b]	0.049
Body weight at 70 d, kg	21.1 [a]	26.1 [b]	< 0.001
Final body weight, kg	106.5	109.7	>0.05
Age at sale, d	185 [a]	180 [b]	< 0.001
Age adjust at 100 kg, d	162 [a]	153 [b]	0.025
Nursery ADG, kg	0.301 [a]	0.372 [b]	< 0.001
Finisher ADG, kg	0.764 [a]	0.816 [b]	0.023
Wean to finish ADG, kg	0.619 [a]	0.657 [b]	0.024

[a,b] Means with different literal for type of vaccine were statistically significant (P<0.05); ADG=Average daily gain; Sc = *Salmonella choleraesuis* ; Li = *Lawsonia intracellularis*

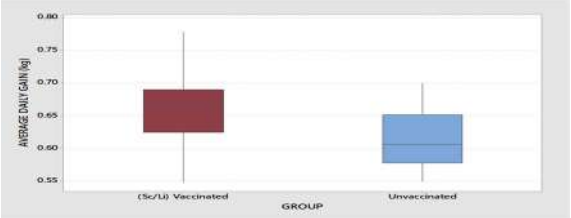

Figure 1. ADG (wean to finish)

Conclusions and Discussion

Under the conditions of this study, average daily gain, body weight at 70 days and body weight at sale were better in the pigs vaccinated with oral live attenuated vaccine (*L. intracellularis/ S. choleraesuis*) than for the non-vaccinated pigs. This result impact directly on the productive performance, obtaining a better benefit cost ratio and return on investment in the vaccinated pigs.

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Comparative study to evaluate the efficacy of tylvalosin and tiamulin for the treatment of *Mycoplasma hyopneumoniae* in a commercial pig farm

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Introduction

Mycoplasma hyopneumoniae (Mhp) is the etiological agent of enzootic pneumonia and one of the most important primary pathogens of the porcine respiratory disease complex (PRDC). The infection is highly prevalent around the world, causing non-productive cough and pneumonia in the cranial lung lobes (1,2,3). In addition, the infection leads to reduced performance parameters, resulting in significant economic losses.

The objective of this study was to compare the efficacy of tylvalosin (Aivlosin® 625 mg/g Water Soluble Granules; ECO Animal Health Ltd.) with tiamulin (Denagard® 45% soluble powder; Elanco) for the treatment of Mhp in a group of naturally exposed pigs and to compare production parameters in the two groups.

Materials and Methods

The trial was performed in a 1,000 sow farm located in Argentina and known to be Mhp positive. A total of 4,000 mixed sex pigs were randomly assigned to one of two treatment groups: Group A which received 5 mg tylvalosin/kg BW/day for 5 consecutive days in drinking water or Group B which received 15 mg tiamulin/kg BW/day for 5 consecutive days in drinking water. Both groups were medicated at 30, 70 and 100 days of age. Medicated water was prepared daily with no other source of drinking water until medicated water was exhausted.

Each treatment group of 2,000 pigs was housed in 4 groups of approximately 500 animals. All pigs had *ad libitum* access to feed and water throughout the trial. Diets were formulated to be identical across treatments. Mortality, average daily gain, feed conversion ratio and days to slaughter were measured. Lungs were evaluated at the slaughterhouse using the Christensen method (4). A total of 1,222 lungs were evaluated, 787 for the tylvalosin group and 435 for the tiamulin group. The Student´s t-test was used for statistical analysis.

Results

The tylvalosin group had better results compared to the tiamulin group for mortality (2.05% vs 2.22 %), days to slaughter (167.6 vs 169.3), final weight (114.6 kg vs 114.3 kg) and average daily gain (0.884 kg vs 0.866 kg) though these differences were not statistically significant. A statistically significant difference was observed for feed conversion rate, with the tylvalosin group more efficient than the tiamulin group (2.72 vs 2.87). The percentage of affected lungs with type A, B and C lesions was higher in the tiamulin group than in the tylvalosin group (10.8% vs 1.72%; 14.71% vs 12.71% and 5.06% vs 4.57%, respectively).

Table 1. Health and Production Parameters

Group	Tylvalosin	Tiamulin
Mortality (%)	2.05	2.22
Days to Slaughter	167.6	169.3
Final Weight (kg)	114.6	114.3
ADG (kg)	0.884	0.866
FCR	2.72[a]	2.87[b]
Affected lungs (%)	28.08	31.49
Type A lesion (%)	1.72	10.8
Type B lesion (%)	12.71	14.71
Type C lesion (%)	4.57	5.06

°Superscripts indicate statistically significant differences ($p \leq 0.05$).

Conclusion and Discussion

In this study, tylvalosin was more effective than tiamulin in reducing mortality and lung lesions in pigs naturally infected with Mhp and in recuperating production losses that are associated with enzootic pneumonia.

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Vaccination of gestating sows against *M. hyopneumoniae* – a useful adjustment to piglet "only" vaccination?

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Introduction

Although vaccination against *M. hyopneumoniae (M. hyo)* is widely applied worldwide, this infectious agent is still leading to bronchopneumonia and therefore to high economic losses in swine production (1). This study investigated *M. hyo* colonization at weaning, *M. hyo* antibody leves in sows and piglets and lung lesions at slaughtering of pigs from *M. hyo* vaccinated (whole herd sow vaccination vs. sow vaccination 3-weeks prior to farrowing) and non-vaccinated sows.

Materials and Methods

The following three vaccination schemes were tested consecutively on a commercial pig farm in western Germany, in which respiratory diseases were seen in rearing piglets and fattening pigs:

Table 1. Overview of the vaccination schemes

	1	2	3
sows	No vaccination	Whole Herd vaccination (Ingelvac MycoFLEX®)	Vaccination 3 weeks ante partum (Ingelvac MycoFLEX®)
piglets	Two-Shot vaccination at day 5 and 26.	One-Shot vaccination at day 21 (Ingelvac MycoFLEX®)	One-Shot vaccination at day 21. (Ingelvac MycoFLEX®)

Sows in vaccination scheme 3 had already been vaccinated during whole herd vaccination 6 month earlier. Blood samples of sows (15 sows per vaccination scheme) and two piglets per litter were collected at the end of lactation period (d 26) to determine the *M. hyo* antibody status (sample-to-positive (S/P) ratio) (IDEXX M.hyo Ab ELISA, IDEXX GmbH, Ludwigsburg). Bronchoalveolar Lavages (BALFs) were taken from the piglets to be tested for *M hyo*-antigen (2). Thereafter, lung lesions of these piglets were scored at the slaughterhouse.
Differences between the groups were tested using the t-test (normal distributed) and the Wilcoxon-test (not normal distributed data; significance level: $p<0.05$). Qualitative variables such as the presence of M. hyo in Lavages were tested using Chi-Quadrat-homogeneity of variance by Pearson (SAS® Enterprise Guide 4.3, Cary, NC, USA).

Results

M. hyo antigen was detected in 38.5^{b} % of the BALFs taken from piglets from non-vaccinated sows. The detection rate decreased significantly after the introduction of sow vaccination (scheme $1:0^{a}$ % vs. scheme 2: 6.67^{a} %). *M. hyo* Antibody levels of sows were higher in both vaccinated groups and significantly higher in the group vaccinated prior to farrowing (S/P ratio $0.50^{b}\pm0.31$ vs. $0.81^{b}\pm0.49$ vs. $1.17^{a}\pm0.30$; $p=0.0007$). In addition to that, the ratio of sows in which *M. hyo* antibody levels could be detected increased most in the group vaccinated ante partum (77.8% vs. 78.6% vs. 100%). Antibody levels of piglets altered parallel to the sow's. Piglets from vaccinated sows had significantly higher antibody levels at weaning than piglets from unvaccinated sows ($0.16^{b}\pm0.12$ vs. $0.55^{a}\pm0.40$ vs. $0.71^{a}\pm0.30$). Ratio of *M. hyo* antibody positive piglets increased from 6.67^{b} to 58.3^{a} to 80^{a} %. Lung lesions at slaughtering decreased significantly after sow vaccination from 28.9% to 17.7%.

Conclusions and Discussion

Early colonization with *M. hyo* can already be seen in suckling piglets.
Sow-vaccination against *M. hyo* can help to reduce colonization-rate of piglets at the time of weaning. Together with vaccination of piglets the implementation of a sow vaccination-program can be a useful tool to control *M. hyopneumoniae*-infections in herds with respiratory disease.

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Occurrence of gastrointestinal diseases in wild boars before emergence of African swine fever in Poland

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Introduction

Clostridium perfringens is a gram-positive, spore-forming bacteria that may cause a variety of toxic-specific lesions and gastrointestinal diseases in pigs and wild boars. Enterotoxigenic *Escherichia coli* (ETEC) is a type of *E. coli* and one of the leading bacteria causing diarrhea in swine worldwide. *Lawsonia intracellularis* and *Brachyspira hyodysenteriae* are the most common bacterial diarrheal diseases. The aim of this study was to investigate the prevalence of cpa and cpb2-positive *C. perfringens*, enterotoxigenic *Escherichia coli* (ETEC) and proliferative enteropathy caused by *Lawsonia intracellularis* and swine dysentery with the etiological agent *Brachyspira hyodysenteriae* in hunted wild boars from the territory of Poland.

Materials and Methods

In total 203 samples of faeces from wild boars were collected between 2011 and 2013 years. The samples were cultured on Columbia agar plates for *C. perfringens* and agar with 5% horse blood for *E.coli.* The typical colonies surrounded by characteristic haemolysis zone have been observed after overnight incubation at 41°C for *C. perfringens* and at 37° C for *E.coli.* Next, the crude whole single colony lysate was used in multiplex PCR assays targeting virulence toxin genes cpa, cpb (β-toxin gene) and cpb2 for *C. perfingens* and toxin genes F4, F18 and Stx2e for *E.coli.* All of received faecal samples were examined for the presence of *L. intracellularis* and *B. hyodysenteriae* by real – time PCR assay.

Table 1. *C.perfringens* occurrence in samples of faeces from wild boars in Poland.

C. perfringens	cpa	cpb2
Number of samples	18	9
Total %	8,8%	4,4%

Table 2. E. coli occurrence in samples of faeces from wild boars in Poland.

E.coli	F18
Number of samples	31
Total %	15%

Table 3. *L. intracellularis* occurrence in samples of faeces from wild boars in Poland

L. intracellularis	Positive samples
Number of samples	6
Total %	3%

Results

On the basis of the conducted study in 27 (8.8%) out of 203 tested samples the presence of *C. perfringens* have been confirmed by PCR specific to cpa gene. Interestingly, in 4.4% examined wild boars the presence of cpa and cpb2 amplicons have been confiremd. In 15 percent of *E.coli* isolates the sole enterotoxin F18 gene has been identified. In case of *L. intracellularis* only three percent of tested wild boars were positive. None of samples were positive for the *B. hyodysenteriae* presence.

Conclusions and Discussion

The study showed that wild boars in Poland might be the reservoir of ETEC, *C. perfringens* and *L. intracellularis*. Bearing in mind that the analysed bacteria may circulate among wild boars in environment may facilitate the real assumption of epidemiological threat for pig herds located close to the area of forests and other ecological niches with dense population of wild boars.

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Post-mortem findings in sows - What are the principal microbiological agents involved in septic locomotor disorder?

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Introduction

In the last decades it was observed a large genetic improvement in swine females' selection, fact that associated with the intensification of the production system has been resulting in the improvement of pig industry productivity. However, the increase in mortality rates in sows is becoming a new critic point of the production for the swine herd. Sow mortality has been growing in several countries (1,2,3), causing economic loss due to replacement and opportunity costs (3). The infectious causes of locomotor disorders (arthritis, muscular abscesses, osteomyelitis and suppurative myositis) can be responsible for 27 % of all deaths (2), and are also considered as secondary lesions, contributing to sows death (1).

Despite the high impact of septic locomotor disorders, there are few studies about sows mortality that performed a microbiological isolation and identification of the agents involved that can contribute to the increase in sow mortality (1,2). Therefore, the objective of the present study was to determine the principal microbiological agents involved in infectious locomotor disorders from deceased sows.

Materials and Methods

It was performed necropsy in 33 sows that died with clinical symptomatology as the inability to stand up. Samples were collected from 19 articular fluids and 18 muscular abscesses, totalizing a total of 37 samples. The samples were collected with the aid of sterile needle, syringe and swabs with culture medium. The samples were plated and incubated in aerobiosis in MacConkey Agar and Blood Agar, and in anaerobiosis in Brucella Agar. All samples were incubated at 37° C for 24/48 hours. The isolated colonies were identified by matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS).

Results

It was observed bacterial growth in all females/samples (356 bacterial colonies selected). It was observed mixed infection (two or more agents) in 70.27% (26/37) of the cases; from these mixed infection, it was verified that 3 agents were present in 27.03% (10/37); 4 agents in 10.81% (4/37) and five or more in 10.8% (4/37). The principal agent was *Trueperella pyogenes* that was presented in 70% of the septic locomotor disorders; the remaining bacterial agents isolated can be visualized in figure 1.

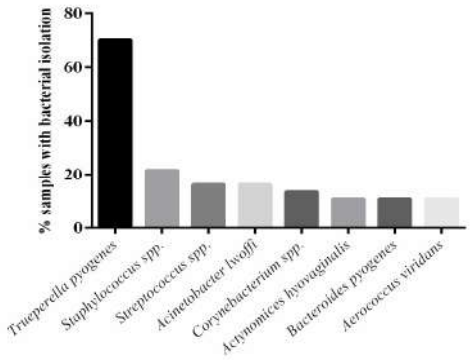

Figure 1. Percentage (%) of microbiological species isolated from locomotor disorder from death sows

Conclusions and Discussion

The septic locomotor disorders have a great impact on sows mortality (1,2). The females with septic locomotor disorders presents reduced the feed intake and water consumption, these facts causes a catabolic state and increases the chance of opportunistic infections. Cachectic females due locomotor disorders are euthanized more frequently, increasing the mortality rate of the herd (1). *T. pyogenes* is an emerging clinical, epidemiological, and economic problem for swine production, because its infection usually results in the necessity of the elimination of infected animals and the discard of carcasses with suppurative lesions at slaughterhouses. *T. pyogenes* may cause infection as a primary etiological agent, but more frequently this species is involved in polymicrobial diseases and was already isolated from arthritis in dead sows (1). Despite this there is few information about the bacterial interaction in septic locomotor disorders. In conclusion, septic locomotor disorders can be result of polymicrobial disorders, which *T. pyogenes* plays an important role. Further studies are essential to understand the interaction between the microorganisms in septic arthritis.

Acknowledgments

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Hemothropic mycoplasmas infection associated with productive impact in fattening pigs from Brazil

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Introduction

Hemotropic mycoplasmas (HMs) are associated with infectious anemia in pigs. Three microorganisms have been officially identified as Porcine Hemoplasmas (PH), namely such as *Mycoplasma suis*, *Mycoplasma parvum, and Mycoplasma haemosuis* (9). *Mycoplasma suis* is the main agent associated with the disease, which is known as swine hemoplasmosis, a possible cause of economic losses worldwide. HMs have been described in commercial and non-commercial pig farms in Brazil (1,5,6), China (7,8) and Germany (2,4), countries in which the production of these animals is economically important. In addition to *M. suis*, *M. parvum* has already been detected in Brazil, and it has been reported as a non-pathogenic bacterium in pigs (9). Thus, this work aimed to perform the detection and quantification of HMs in finishing pigs, and well as its correlation to average daily weight gain (ADWG).

Materials and Methods

Whole blood samples from 318 pigs (174 females and 144 males) were collected from a commercial finishing farm. The first collection was carried out at 65 days old (d0), when the animals entered the finishing phase, and the second collection was carried out 105 days later (d105) at 170 days of age, when they left for slaughter. In addition, the animals were weighed in both dates: d0 and d105.

Blood samples were stored in liquid nitrogen and then transferred to the -80ºC freezer until processing. DNA extraction was performed following a previously described protocol (3,5). DNA samples were submitted to conventional PCR targeting the *gapdh* gene in order to avoid false negatives results in quantitative real-time PCR (qPCR). Then, the *gapdh*-positive DNA samples were submitted, in duplicate, to qPCR test for HMs based on the 16S rRNA gene (1).

Pearson and Spearman correlation tests were performed in order to investigate the potential associations between ADWG and the quantification values of the HM-16S rRNA fragments detected at d0 and d105.

Results and discussion

Out of the 318 samples, 190 were positive on d0 and 304 were positive on d105, indicating that 95.6% of the animals collected at the slaughterhouse were positive for HMs. These results corroborate with recent studies reported in Brazil (5,6,9). The overall occurrence was 37% (120/318), being 43% in females (75/174), and 31% in male pigs (45/144). While eight animals were negative in both blood sampling, six animals were positive on d0 and negative on d105, which indicated that the immune system might have controlled the infection at an earlier phase.

Quantification values (SQ) ranged from $1.07x10^0$ to $4.30x10^6$ HM 16S rRNA copies/µL on d0 and from $1.41x10^0$ to $1.16x10^7$ copies/µL on d105, indicating that the animals were chronically infected at d0 (9), and that bacteremia increases as time goes by.

On Spearman correlation test, no significant results were observed between ADWG and SQ at d0 ($p > 0.05$), which could be explained by the chronic stage of the disease, indicated by the low bacteremia. However, when comparing ADWG with SQ at d105, a significant correlation was observed ($p < 0.05$) but with low coefficient value (0.18), indicating that the two variables are weakly correlated, what makes us to believe that there were other associated factors, such as respiratory diseases, contributing to the decrease of DWG.

In addition, the use of antimicrobials in feed until d90 (15 days before slaughter) could have controlled the disease and its productive impact. Since antimicrobial use in pig production is a big concern, many countries have prohibited its use as growth promoters and disease prevention, allowing pathogens, such as HMs, to proliferate and cause disease. Therefore, diseases inhibited by the preventive use of antimicrobials could emerge and become a problem in pig production worldwide.

Conclusions

The results showed that MHs circulate in commercial pig farms and that the infection is potentially involved in ADWG losses in infected animals.

Acknowledgments

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An overview of bacterial reproductive tract infections of sows housed in Brazilian farms

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Introduction

Purulent vulvar discharges are often related to reproductive tract infections, most often characterized as metritis, which can decrease sow fertility, cause abortions and lead to a reduction in the number of piglets born and farm profitability (1, 2, 3). There is still little information about the bacterial agents involved in these infections in Brazilian herds, as well as other epidemiological data. Therefore, due to the economic impact on swine production, the aim of this study was to identify the main bacterial agents involved in these infections and to bring new information about the affected sow population.

Materials and Methods

One hundred and ninety-seven sows with purulent vulvar discharge were sampled at four commercial farms in the states of Minas Gerais, Paraná, São Paulo and Mato Grosso. Swabs from the deep vaginal canal were collected using a sterile speculum to prevent fecal contamination during passage through the vulvar vestibule. The samples were seeded in MacConkey, Columbia and Brucella agar incubated in aerobiosis and anaerobiosis at 37°C for 24 to 48 hours. The isolated colonies were identified by matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS).

Results

All samples were positive for bacterial isolation. Over 100 species were identified within 50 genera. The ten most isolated species in examined sows are shown in table 1. Only 14 animals (7.1%) yielded pure culture (a single isolated species), while 183 animals (92.9%) yielded mixed cultures. The mean number of different species isolated from an animal was 3.3±1.5, and the maximum was eight (figure 1). Sows of various parity orders were sampled, being the parity order mean 3.4±2.5. Most females (39.1%) with vulvar discharge belonged to parity 1 and 2. Vulvar discharges were more frequent in sows between weeks 1 and 3 after farrowing and week 1 after artificial insemination (AI) (figure 2).

Conclusions and Discussion

Vaginal swabs of sows with purulent vulvar discharge showed great bacterial richness, which makes the diagnosis of the infection agent difficult. Isolation of bacteria such as *Escherichia coli*, *Streptococcus suis* and *Enterococcus faecalis* at high frequencies reinforces other studies indicating that urogenital infections are closely related to fecal microbiota agents (4). Infections after farrowing and AI were more frequent. This is in line with previous studies (5) suggesting these phases as risk factors and that more attention should be given to sows' management in these stages. Due to the complexity of bacterial population in samples of sows with vulvar discharge, other approaches, such as metagenomics, should be employed to assess the vaginal microbiome, which may provide a better understanding of bacterial compositions in the reproductive tract infections of sows.

Table 1. Most frequently recovered bacterial species from sows with purulent vulvar discharge.

Agent	Positive sows (%)
E. coli	53,3
Streptococcus suis	26,4
Streptococcus dysgalactiae	20,8
Staphylococcus hyicus	19,3
Enterococcus faecalis	14,2
Proteus mirabilis	11,2
Actinomyces hyovaginalis	10,7
Streptococcus canis	10,2
Trueperella abortisuis	10,2
Corynebacterium diphtheriae	9,1

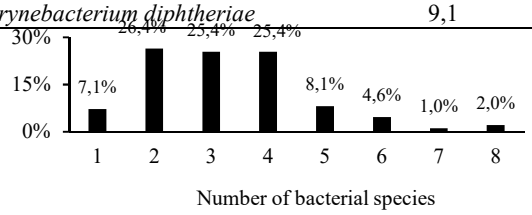

Figure 1. Frequency of animals with pure or mixed cultures.

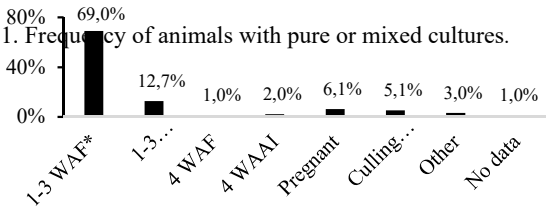

Figure 2. Frequency of sows with vulvar discharge by cycle phase. *Weeks after farrowing; **Weeks after artificial insemination.

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Characterization of *Corynebacterium* species isolated from diseased and asymptomatic swine

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Introduction

The *Corynebacterium* genus is composed of species with toxigenic potential, of which *C. diphtheriae* is one of the most studied (1). *Corynebacterium* species can be constituent of several microbiotas and are often considered contaminants (2). Swine have already been described as carriers of zoonotic species (3). The aim of this study was to characterize *Corynebacterium* species isolated from different isolation sites of diseased and asymptomatic pigs.

Material and methods

A total of 31 *Corynebacterium* strains were analyzed. Strains were obtained from 22 pigs, originated from 10 herds from four Brazilian states, between 2011 and 2017. Of these animals, nine were asymptomatic (nasal swab samples) and 13 presented genitourinary infection or septicemia; from the diseased animals were collected: six vaginal swabs, 13 urines, and one sample of spleen, pericardium and abdominal cavity. Samples were plated on blood agar and incubated at 37ºC for 24h. The *Corynebacterium*-like colonies were initially screened by MALDI-TOF (Matrix Associated Laser Desorption Ionization – Time of Flight) mass spectrometry as described by Hijazin et al. (4) and species identification was confirmed by 16S rRNA sequencing using Twomey et al. (5) primers. The amplicons were purified and sequencing was performed at the Human Genome Research Center (IB-USP, Brazil). The phylogenetic analysis was performed with Mega 10.0.5 software using the maximum-likelihood method and 500 bootstrap replicates for branch support statistical inference. Identified species were displayed in a dendrogram (Bionumerics 7.6) for further origin assessment.

Results

From the studied strains, four *Corynebacterium* species were identified (*C. amycolatum, C. confusum, C. diphtheriae* and *C. phoceense*). The MALDI-TOF MS could not identify only *C. phoceense* as this species is not yet contained in its database. Therefore 17 strains were initially screened as *Corynebacterium* sp. and the species was only identified by sequencing. Interestingly, the *C. phoceense* species presented higher origin heterogeneity (Figure 1). In contrast, *C. diphtheriae* was only detected in sows with purulent vulvar discharge from two distinct herds of Minas Gerais state at different periods. It is also highlighted that three species were detected among the 13 strains isolated from urine samples: *C. phoceense* (38.5%), *C. confusum* (7.7%) and *C. amycolatum* (53.8%) (Figure 1).

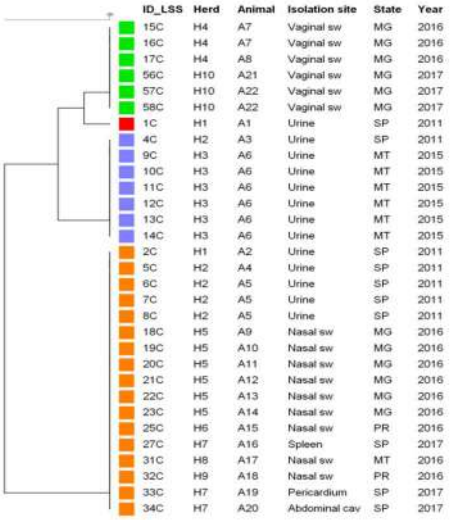

Figure 1. Dendrogram displaying identified *Corynebacterium* strains. Colored blocks correspond to species: green - *C. diphtheriae*; red - *C. confusum*; blue - *C. amycolatum*; orange - *C. phoceense*.

Discussion and Conclusion

The MALDI-TOF MS has already been successfully applied for *Corynebacterium* identification (2). Our results not only corroborate that MALDI-TOF MS is an useful technique for *Corynebacterium* identification, but also demonstrates that the genus present importance for swine health and that swine may be carriers of potential zoonotic species. Boschert et al. (6) also detected several *Corynebacterium* species from asymptomatic pigs and farmer's. The authors reported very similar patterns of the species composition between the pigs and farmers suggesting a potential risk of zoonotic transmission. This demands attention specifically for the risk of dissemination of pathogenic species, such as *C. diphtheriae*.

Acknowledgments

The authors would like to acknowledge the financial support provided by FAPESP (grants 2018/00514-8, 2016/25745-7).

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Distribution and occurrence of *Lawsonia intracellularis* in Brazil

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Introduction

Lawsonia intracellularis is an enterocyte-infecting bacterium that causes porcine proliferative enteropathy (PPE), a chronic disease characterized by thickening of intestinal mucosal mostly at the ileum (1). The acute presentation of PPE is known as proliferative hemorrhagic enteropathy (PHE) and the chronic presentation as porcine intestinal adenomatosis (PIA) (1). Subclinical presentation appears when animals have usually underrated growth but still are excreting bacteria serving as a silent reservoir (2). Unfortunately, diarrhea is not a pathognomonic clinical sign and resembles to other enteric diseases. Therefore, a fine molecular detection such as quantitative PCR is necessary to identify positive animals and survey pig farms. Here we present the occurrence and distribution of *L. intracellularis* in Brazilian swine farms.

Materials and Methods

The molecular detection of *L. intracellularis* was conducted by real time PCR (qPCR) using faecal samples. A total of 53 different farms of grower/finisher pigs with frequent episodes of diarrhea were included in this study. At the farm, stool samples (n = 15 each indicated age) were collected from 60, 90, 120, 150 and 170 days-old non-vaccinated animals. The stools were collected directly from the rectal ampoule and sent refrigerated to the laboratory. Then, each fecal sample was diluted to 10% in PBS (3) and used for genomic DNA extraction using MagaZorb DNA Mini-Prep Kit (Promega, USA) according to manufacturer's protocol. The molecular detection and quantification of *L. intracellularis* was carried out by qPCR as previously described (4). A linearized plasmid containing the target sequence was included as a positive control as well as genomic DNA extracted from an attenuated *L. intracellularis* strain (Enterisol Ileitis, Boehringer Ingelheim, USA). The qPCR cut-off for *L. intracellularis* was set at 40 cycle threshold (Ct). The bacteria load (Ct) data found at different ages were compared with Kruskal-Wallis test.

Results

As illustrated in figure 1, this study included 53 pig farms geographically localized in 5 different states, which represent more than 80% of the national pig production. Most samples were derived from Southern Brazil, followed by Midwest and Northwest. A total of 40 (75.5%) out the 53 farms were molecularly positive for *L. intracellularis*. At day 60, only 7 (13.20%) farms had pigs shedding the bacteria. In contrast, at days 90, 120, 150 and 170, pigs shedding bacteria were found in > 50% of the farms. As indicated in the table 1, the load of *L. intracellularis* on feces was significantly higher at days 90 and 120 compared to days 60, 150 and 170, indicating that this would be a good marker period to sampling pigs for *L. intracellularis* detection.

Figure 1. Geographical distribution of the pig farms sampled.

Table 1. *L. intracellularis* shedding in feces of pigs at different ages. Different letters indicate statistical differences ($p < 0.05$).

Age (days)	Load of *L. intracellularis* (Ct/g)		
	Ct Range	Mean ± SD	
60	32.90 – 38.40	36.09 ± 1.80	a
90	18.10 – 36.20	29.70 ± 3.70	b
120	19.20 – 38.20	31.07 ± 4.09	bc
150	21.40 – 38.30	33.49 ± 4.80	a
170	19.80 – 38.00	33.01 ± 5.80	a

Conclusions and Discussion

Our results show a high percentile of farms with pigs shedding *L. intracellularis*. Most samples were not clinically related to ileitis but were molecularly positive for the bacterium, indicating that the majority of herds evaluated had a significant number of sub clinically infected animals. This data serve as an alert to practitioners and swine producers strengthening the importance of an effective diagnostic method to monitor herd health status and helping to build control and prevention strategies.

Acknowledgments

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Inactivated vaccine reduces *Lawsonia intracellularis* shedding in pigs at field conditions

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Introduction

Lawsonia intracellularis is an obligate intracellular bacterium that causes porcine proliferative enteropathy (PPE), mostly observed in growing and finishing pigs, and characterized by diarrhoea, reduced growth rate and decreased food conversion (1). The use of vaccines is a pivotal strategy to controlling infection and avoiding clinical signs and economical losses. Here, we evaluated the capacity of an inactivated *L. intracellularis* vaccine to reduce bacteria shedding in field condition.

Materials and Methods

The field trials were carried out in two Brazilian commercial pig farms with a history of *Lawsonia*-associated mortality, mainly in pigs with more than 20 weeks of age. The farm A had a farrow-to-finish operation (3,500 sows) and farm B was a grower/finisher production unit (5,000 pigs). These cross-sectional studies included 7,000 pigs from farm A and 5,000 pigs from farm B. In farm A, 3,500 pigs were vaccinated intramuscularly once at 28 days of age in the nursery unit using 2 mL of the inactivated *L. intracellularis* vaccine potentiated with Diluvac Forte Adjuvant (Vac.FA). The remaining 3.500 pigs were not vaccinated (NVac.FA) and kept as control. In farm B, 3,000 pigs were vaccinated with the same vaccine at 42 days of age in the nursery unit (Vac.FB) and the remaining 5,000 pigs were kept as unvaccinated (NVac.FB) controls. At day 70, pigs were transferred from the nursey unit to farm B. Stool samples were randomly collected from 15 animals per group at different ages (30, 60, 90, 120 and 140 days) aiming *L. intracellularis* quantification by qPCR assay (2). During the experiment (grower/finisher phase) animals from both farms received antibiotic in feed according to the prophylactic antibiotic strategy used in the farm. The results are presented as the number of positive animals for *L. intracellularis* in feces and their relative load of infection.

Results

The effective prevention of the clinical manifestation of *L. intracellularis* infection in pigs can be achieved by vaccination. As illustrated in figure 1, all stool samples were negative to *L. intracellularis* until day 60; however, at day 90, all non-vaccinated pigs from Farm A and one from Farm B (Fig.1-i and ii) were shedding the bacteria in the feces. At this moment, *L. intracellularis* could not be detected in the feces of vaccinated animals (Vac.A and Vac.B). At day 120 and 140 the bacteria shedding decreased in pigs from farm A and increased in pigs from farm B in the NVac.FA and NVac.FB groups. In farm B, just two vaccinated pigs were shedding *L. intracellularis* at day 120; in farm B we detected 5 pigs. The load of *L. intracellularis per* gram of feces is represented in figure 1-iii and iv and highlights that 33% of the animals from NVac.FA group had a high *L. intracellularis* load at day 90 (Ct < 20) which is compatible with proliferative lesions [3] that can compromise the absorption of nutrients by enterocytes cell. The relative cut-off (*ct*) value detected in vaccinated animals at day 120 were > 30 in Vac.FA and > 36 in Vac.FB groups, showing that the vaccine is not capable to completely prevent *L. intracellularis* infection but, most importantly, it significantly reduces bacterial shedding on feces which, in turn, indicates a low level of histological alteration on mucosal tissue.

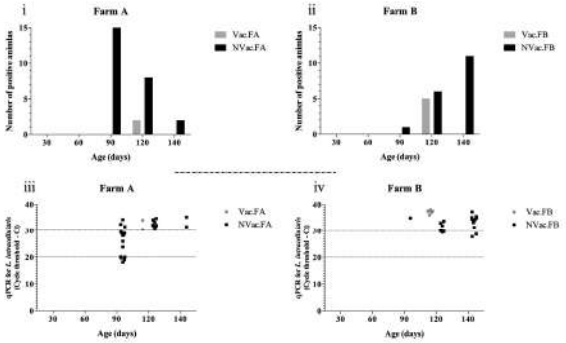

Figure 1. Number of positives animals for *L. intracellularis* (15 animals/group/age) (i and ii) and qPCR Ct values detected in feces from vaccinated and non-vaccinated pigs (iii and iv).

Conclusions and Discussion

In the present study, we demonstrated that an inactivated *L. intracellularis* vaccine, administered intramuscularly once in piglets with 28 or 42 days-old, is capable to significantly reduce *L. intracellularis* shedding in feces.

Acknowledgments

This work was financially supported by MSD.

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Preliminar retrospective study of the serological behavior of *Lawsonia intracellularis* in swine intensive production system in Chile

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Introduction

Lawsonia (L) intracellularis is a disease, producing a condition known as proliferative enteritis (1, 2). This disease generates a lot of economic losses around the world, because it reduces the efficiency of food conversion (3). Serology can be used to identify infected herds; passive immunity drops and seroconversion time. However, finding high antibody titers only indicates exposure to this bacterium (4, 5). In South America there is little information on the prevalence and serological behavior of this disease. The main objective of this study is to determine the prevalence and serological behavior of *Lawsonia intracellularis.*

Materials and Methods

In order to do that the ELISA serological results of *L intracellularis* will be analyzed, from samples of 23 swine production farms in Chile (15 multisite and 8 monosite), carried out during the years 2013 to 2018 in the Swine Diagnostic Laboratory of the University of Concepcion, Chile).

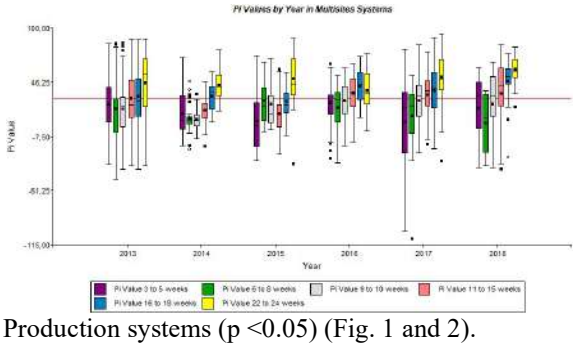
Fifty-two serological profiles in total (n = 1947), corresponding to the Commercial Line, of different age were analyzed. The samples were classified in A: 3 to 5 weeks (purple); B: 6 to 8 weeks (green); C: 9 to 10 weeks (grey); D: 11 to 15 weeks (red); E: 16 to 18 weeks (blue); F: 22 to 24 weeks (yellow). Additionally, the production system was classified as monosite and multisite.

The ELISA test used was Svanovir® *L intracellularis* / Ileitis-Ab, following the standardized protocol by the manufacturer (Svanova®).

The data was analyzed using by ANOVA, and if it was not normal by the Kruskal-Wallis test.

Results

A prevalence of 49.46% between 2013 and 2018 was found. A significant difference between monosite and multi-site systems between years was observed. Serological profile behaves different between year and



Production systems ($p < 0.05$) (Fig. 1 and 2).

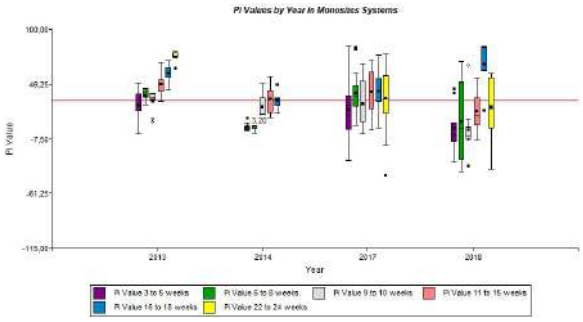


Figure 2: Box and whisker diagram of Pi values, measured by ELISA, to *L intracellularis* of 8 monosite swine farms, classified by group of age and year.

Conclusions and Discussion

Different types of behavior can be observed between years. Multisite system (Fig. 1) lean to have high Pi values due to the passive immunity, than the monosite system. Then the Pi values decrease as it starts to drop the maternal antibodies and after this increase constantly as seroconversion starts to rise. In 2016, the serological profile was a little different, because, there is an early increase of the Pi values with a subsequent decrease and then a late seroconversion. In 2017, the Pi values tends to increase constantly after the 6 to 8 weeks.

On the other hand, monosite systems (Fig. 2) there were no samples in 2015 and 2016. In 2013, 2014 and 2017, the serological profiles behave like 2017, but in 2014 and 2017 a little decrease was observed in the last samples age. In the year 2018, there is an irregular serological pattern, where there is not a clear trend in the Pi values.

The data suggest that the passive immunity drops around 9 to 10 weeks of age, and seroconversion starts around the 11 to 15 weeks of age. Seropositivity in monosites, without regard to the passive immunity, is 58,0% and in multisites is 55,5%. In this study, al the farms were positive, so farm prevalence is 100%.

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Retrospective study of the serological behavior of *Lawsonia intracellularis* in sows and gilts in swine intensive production system in Chile

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Introduction

Porcine proliferative enteropathy (PPE) is a disease that causes diarrhea, slow growth in growing pigs and hemorrhagic diarrhea and sudden death in adults (1). This disease is caused by an intracellular bacterium called *Lawsonia intracellularis* (2). This disease generates a lot of economic losses around the world, because it reduces the efficiency of food conversion (3). It is known that an important risk factor to consider are the sows and gilts in breeding herds, despite of vertical transmission has not been described before (4). The aim of this study was to determine the seroprevalence of *L. intracellularis* in sow and gilts in breeding herds in Chile using ELISA.

Materials and Methods

Samples from 2013 to 2018 were analyzed by ELISA (Svanovir® *L intracellularis* / Ileitis-Ab, following the standardized protocol by the manufacturer (Svanova®)) in the Swine Diagnostic Laboratory of the University of Concepcion, Chile). The samples come from 18 unvaccinated swine production systems from Chile of which 12 were from sows and 6 from gilts breeding herds. Twenty serum profiles from sows (n = 584) and eight profiles from gilts (n = 407) of different parturition and ages were analyzed. The samples were classified by parity in P0, P1, P2 to P3, P4 to P5 and P6 or more. Besides, gilts were classified in A: 3 to 5 weeks; B: 6 to 8 weeks; C: 9 to 10 weeks; D: 11 to 15 weeks; E: 16 to 18 weeks; F: 19 to 20 weeks; G: 22 to 24 weeks; H: 26 to 30 weeks and I: 32 weeks.

To determinate the seroprevalence in gilts, ranges from D to I were analyzed to avoid passive immunity interference.

The data was analyzed using ANOVA.

Results

An overall seroprevalence of 73,3% between 2013 and 2018 was observed, 83,2% and 54,5% corresponds to sows and gilts, respectively (Table 1 and 2). Significant difference between both categories was observed ($p < 0.05$). Sows present an evident increase of the seroprevalence as they increase the number of parities, due to the exposure time to these bacteria

Conclusions and Discussion

As the results of this study showed, the prevalence of *L intracellularis* is relatively high (Table 1). Having the highest values of seroprevalence in this study in comparison with gilts. This is concordant with the previous studies by Jacobson *et al* (2010)

These results suggest that as the sows have been exposed to the pathogen, they will transfer the antibodies to their offspring, based in the presence of seropositive gilts between three to nine weeks of age.

Table 1. ELISA results of the sows from 12 breeding herds from Chile.

Range	Pos (%)	Susp (%)	Neg (%)
P0	74.6	7.7	17.6
P1	82.4	5.9	11.8
P2 a P3	83.8	4.6	11.5
P4 a P5	85.3	3.9	10.9
P6 o más	95.1	4.9	0.0
Total	83.2	5.5	11.3

In the case of the gilts an increase of seropositivity from range d to e, but then a sudden decrease in range f. After this in range g to i there is a marked upward trend in seropositivity (Table 2). According to these results, suggest that the main risk of this bacteria occurs in the breeding gilts, due to the high seropositivity in the last ranges, that are similar to what was registered in sows in parity 6 or more. This condition as Jacobson, et al. is a very important risk factor in the upsurge of the clinical presentation.

These results were from breeding herds that were not using vaccines. Nowadays there is a new inactivate vaccine from Merck Animal Health. So, according to this, is important to study de how this vaccine affects the risk factors associated with the gilts and sows and what is the role in protection to *L intracellularis*.

Table 2: ELISA results of the gilts from 6 breeding herds from Chile.

Range	Pos (%)	Susp (%)	Neg (%)
d	32.3	12.3	55.4
e	54.5	14.5	30.9
f	36.0	36.0	28.0
g	56.9	24.6	18.5
h	66.3	18.8	15.0
i	95.0	5.0	0.0
Total	54.5	18.4	27.1

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Retrospective study of the serological behavior of *Lawsonia intracellularis* in swine intensive production system in Chile

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Introduction

Lawsonia (L) intracellularis is a disease, producing a condition known as proliferative enteritis (1, 2). This disease generates a lot of economic losses around the world, because it reduces the efficiency of food conversion (3). Serology can be used to identify infected herds; passive immunity drops and seroconversion time. However, finding high antibody titers only indicates exposure to this bacterium (4, 5). In South America there is little information on the prevalence and serological behavior of this disease. The main objective of this study is to determine the prevalence and serological behavior of *Lawsonia intracellularis.*

Materials and Methods

In order to do that the ELISA serological results of *L intracellularis* were analyzed, from samples of 23 swine production farms in Chile (15 multisite and 8 monosite), carried out during the years 2013 to 2018 in the Swine Diagnostic Laboratory of the University of Concepcion, Chile).

Fifty-two serological profiles in total (n = 1947), corresponding to the Commercial Line, of different age were analyzed. The samples were classified in A: 3 to 5 weeks (purple); B: 6 to 8 weeks (green); C: 9 to 10 weeks (grey); D: 11 to 15 weeks (red); E: 16 to 18 weeks (blue); F: 22 to 24 weeks (yellow). Additionally, the production system was classified as monosite and multisite.

The ELISA test used was Svanovir® *L intracellularis* / Ileitis-Ab, following the standardized protocol by the manufacturer (Svanova®).

The data was analyzed by ANOVA, and if it was not normal by the Kruskal-Wallis test.

Results

A prevalence of 49.46% between 2013 and 2018 was found. A significant difference between monosite and multi-site systems between years was observed. Serological profile behaves different between year and Production systems ($p < 0.05$) (Fig. 1 and 2).

Conclusions and Discussion

Different types of behavior can be observed between years. Multisite system (Fig. 1) lean to have high Pi values due to the passive immunity, than the monosite system. Then the Pi values decrease as it starts to drop the maternal antibodies and after this increase constantly as seroconversion starts to rise. In 2016, the serological profile was a little different, because, there is an early increase of the Pi values with a subsequent decrease and then a late seroconversion. In 2017, the Pi values tends to increase constantly after the 6 to 8 weeks.

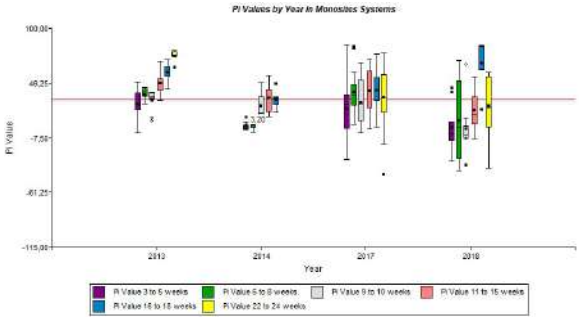

Figure 1. Box and whisker diagram of Pi values, measured by ELISA, to *L intracellularis* of 15 multisite swine farms, classified by group of age and year.

On the other hand, monosite systems (Fig. 2) there were no samples in 2015 and 2016. In 2013, 2014 and 2017, the serological profiles behave like 2017, but in 2014 and 2017 a little decrease was observed in the last samples age. In the year 2018, there is an irregular serological pattern, where there is not a clear trend in the Pi values.

The data suggest that the passive immunity drops around 9 to 10 weeks of age, and seroconversion starts around the 11 to 15 weeks of age. Seropositivity in monosites, without regard to the passive immunity, is 58,0% and in multisites is 55,5%. In this study, al the farms were positive, so farm prevalence is 100%.

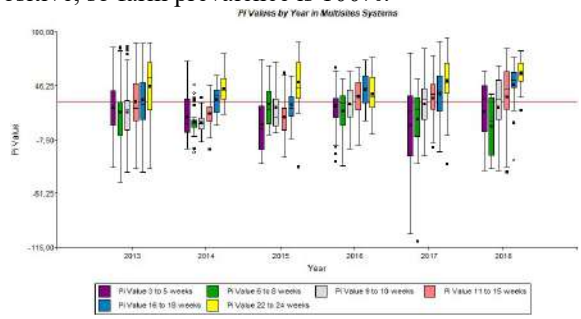

Figure 2. Box and whisker diagram of Pi values, measured by ELISA, to *L intracellularis* of 8 monosite swine farms, classified by group of age and year.

References

1. McOrist et al. 1995. Int J Syst Bacteriol, 45, 820-825.
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3. Collins, A., 2013. Agriculture 3, 536-555.
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Serological behavior of *Mycoplasma hyopneumoniae* in swine intensive production systems from Chile

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Introduction

Mycoplasma hyopneumoniae (Mhyop) is one important pathogen involve in the respiratory diseases complex of growing to finishing pigs. These bacteria have a significant economic impact, due to treatment, vaccination, decreased performance, and the mortality derived from secondary infections (1). Mhyop is worldwide distributed and is characterized by high morbility and low mortality (2). ELISA is the most used method for herd surveillance, reporting a seroconversion 21 days post infection, under experimental conditions, nevertheless it cannot differentiate maternally derived antibodies, vaccination antibodies or antibodies elicited by infection (3, 4). Few studies have been reported about seroprevalence and serological behavior from Chile. The aim of this study was to determine the serological behavior of the *Mycoplasma hyopneumoniae* during the latest years.

Materials and Methods

Mhoyp ELISA serological results from samples of 27 swine production farms from Chile, carried out during the years 2015 to the first quarter of 2020, in the Swine Diagnostic Laboratory of the Universidad de Concepcion, Chile, were compared and analyzed

Ninety-nine serological profiles in total (n = 5343 serum samples), corresponding to the commercial line, of different age were study. The samples were classified in A: 1 to 5 weeks; B: 6 to 7 weeks; C: 8 to 10 weeks; D: 11 to 14 weeks; E: 15 to 18 weeks; F: 19 to 22 weeks; G: 23 to 25 weeks.

Samples were analyzed by the commercial HerdChek® Mhyo Antibody Test Kit (IDEXX Laboratories, Westbrook, ME, USA), following the standardized protocol by the manufacturer.

The data was analyzed by ANOVA with LSD Fisher test and with Kruskal-Wallis when distribution was not normal.

Results

Different behavior by year was observed ($p<0.05$) (Fig. 1), Nevertheless, the passive immunity decays at range B (6 to 7 weeks), and there is a late seroconversion at range F to G (16 to 25 weeks) (Fig. 2).

Conclusions and Discussion

Average serological differences between years were observed ($p<0.05$), with higher values on 2015, and the results suggest a stabilization of the population between 2016 to 2019. The first quarter of 2020 is shown lest variation than the years before (Fig. 1).

The analysis of the results per year considering the ranges of ages (Fig. 2) showed that in 2015 there was a higher passive immunity, that decay later than the years after.

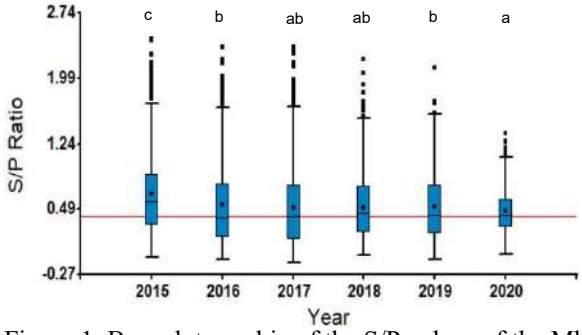

Figure 1. Box-plot graphic of the S/P values of the Mhyop serology of 27 swine farms classified by year. Red line shows the threshold level (S/P ratio of 0.4) for a positive result. [abc] Different letters among years show statistical significance differences ($p<0.05$).

The 2016 serological results, despite they are having higher passive immunity, than in the following years, show an abrupt passive immunity decay at range B.

All years present an increase serological results through range F.

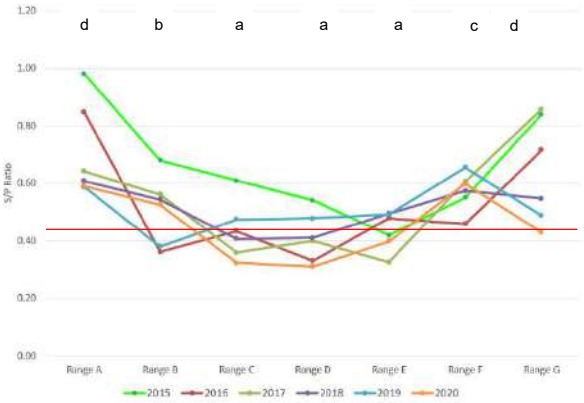

Figure 2. Line chart of the average S/P values of Mhyop's serology by year, classified by age range of 27 swine farms. Red line shows the threshold level (S/P ratio of 0.4) for a positive result. [abcd] different letters show statistically significant differences between average age range, calculated from all the samples for all years in that range ($p<0.05$).

This data suggest that the passive immunity drops around 6 to 8 weeks of age, and a late seroconversion at 19 weeks of age or more.

References

1. Holst, S., 2015. J Swine Health Prod, 23, 321-330.
2. Sibila, M. 2008. Vet J, 181, 221-231.
3. Garza-Moreno, L. 2018. Vet Microbiol, 219, 23-29.
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Seroprevalence of *Mycoplasma hyopneumoniae* in swine intensive production systems from Chile

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Introduction

Mycoplasma hyopneumoniae (Mhyop) is one of the main respiratory pathogens that affect finishing pigs. These bacteria have a significant economic impact, due to treatment, vaccination, decreased performance, and the mortality derived from secondary infections (1). Mhyop is worldwide distributed and is characterized by high morbility and low mortality (2). ELISA is the most used method for herd surveillance, nevertheless it cannot differentiate maternally derived antibodies, vaccination antibodies or antibodies elicited by infection (3). However, latest studies suggest an association between higher serological results and lung lesions in early stages of the disease (4). Few studies have been reported about seroprevalence and serological behavior from Chile. The aim of this study was to determine the seroprevalence of the *Mycoplasma hyopneumoniae* during the latest years.

Materials and Methods

Mhyop ELISA serological results samples of 27 swine production farms from Chile, carried out during the years 2015 to the first quarter of 2020, in the Swine Diagnostic Laboratory of the Universidad de Concepcion, Chile were analyzed.

Three thousand seven hundred and fourty serum samples from the commercial line, of different age were studied. The samples were classified weeks; C: 8 to 10 weeks; D: 11 to 14 weeks; E: 15 to 18 weeks; F: 19 to 22 weeks; G: 23 to 25 weeks. Groups from 3 to 8 weeks were not considered to avoid maternal immunity bias.

Samples were analyzed by the commercial HerdChek® Mhyo Antibody Test Kit (IDEXX Laboratories, Westbrook, ME, USA), following the standardized protocol by the manufacturer.

The data was analyzed by ANOVA with LSD Fisher test and with Kruskal-Wallis when distribution was not normal.

Results

An overall seroprevalence of 50.21% was observed among the years of study. Different seropositive results by year were reported ($p < 0.05$) (Table 1). An increase seropositive results were observed in the latest age ranges (F and G) ($p < 0.05$) (Table 2).

Conclusions and Discussion

In this study, significant differences were found for the seroprevalence results by year, showing the years 2015 and 2019 the highest seroprevalence reported (60.68 and 54.68% respectively), being different among them. On the other hand, the lowest seroprevalence was observed in 2016, which was not statistically different from 2017 and the first quarter of 2020 (Tab. 1).

These results could suggest that a reduction of the seropositivity from 2016 to 2018 this might be related to the control strategies within swine intensive production industry.

In 2019 was a significant increase of seroprevalence and for 2020 up to now there is a reduction in seropositivity values.

Table 1. Percentage of positive, suspect and negative ELISA results of swine samples between 2015 to 2020, classify by years of sampling.

Year	Positive %	Suspect %	Negative %
2015[d]	60.68%	12.88%	26.44%
2016[ab]	44.65%	10.96%	44.39%
2017[a]	44.69%	9.54%	45.76%
2018[b]	48.48%	11.36%	40.17%
2019[c]	54.68%	10.02%	35.30%
2020[ab]	45.32%	18.35%	36.33%
Total	50.21%	12.18%	38.07%

[abcd] Different letters among years show statistical significance differences ($p<0.05$).

Comparing the seroprevalence averages from 2015 to 2020 by age range, there is no high variation except for range F and G, that might suggest seroconversion between 16 to 25 weeks of age, which confirm the late seroconversion of this disease (Tab. 2).

Table 2. Percentage of positive, suspect and negative ELISA results of swine samples between 2015 to 2020, classify by age range.

Age	Positive %	Suspect %	Negative %
Range C[a]	46.15%	12.20%	41.64%
Range D[a]	43.48%	13.15%	43.37%
Range E[a]	43.60%	12.69%	43.71%
Range F[b]	60.74%	9.59%	29.67%
Range G[c]	66.43%	6.95%	26.62%
Total	50.21%	10.92%	37.00%

[abc] Different letters among age ranges show statistical significance differences ($p<0.05$).

All swine intensive production farms were positive to the presence of Mhyop, so farm prevalence was 100. Without regard to the passive immunity the overall seroprevalence was 50.21%.

References

1. Holst, S., 2015. J Swine Health Prod, 23, 321-330.
2. Sibila, M. 2008. Vet J, 181, 221-231.
3. Garza-Moreno, L. 2018. Vet Microbiol, 219, 23-29.
4. García-Morante. 2017. PLoS One, 12, e0175034.

Serological behavior of *Actinobacillus pleuropneumoniae* in Chilean swine intensive production systems

Aline Vera[1], Laura Peralta[1], Álvaro Ruiz-Ormeño[1], Constanza Villarroel[1], Benjamín Céspedes-Cortés[1], Joaquín Álvarez-Norambuena[1], Álvaro Ruiz[1]

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Introduction

Swine pleuropneumonia is a high contagious disease caused by *Actinobacillus pleuropneumoniae* (App), causing significant economic losses worldwide due to mortality and treatment costs (1). There are 18 serovars and 2 biovar of this bacteria (2). Serology is important to understand epidemiology of an App outbreak, among these, ELISA has been described as an easier serological test, automated and the most sensitive and specific (3). Few studies have been done in Chile about the serological behavior of App (4). Therefore, the aim of this study was to determine the maternal immunity duration and time of seroconversion in Chile.

Materials and Methods

App ELISA serological results samples of 18 swine production farms from Chile, carried out during the years 2010 to the first quarter of 2020, in the Swine Diagnostic Laboratory of the Universidad de Concepcion, Chile were analyzed. In this study, year 2019 was not considered due to the low number of samples.

Three thousand nine hundred and ninety-eight serum samples corresponding to the commercial line, of different age were studied. Samples were classified in A: 3 to 5 weeks; B: 6 to 8 weeks; C: 9 to 12 weeks; D: 13 to 16 weeks; E: 17 to 20 weeks; F: 21 to 25 weeks.

Samples were analyzed by a commercial IDEXX APP-ApxIV Ab ELISA Test (IDEXX Laboratories, Westbrook, ME, USA), following the standardized protocol by the manufacturer.

Statistical analysis was made by Kruskal-Wallis as the distribution was not normal.

Results

Different behavior by year was reported ($p < 0.05$) (Fig. 1), Passive immunity decay was observed at range c (9 to 12 weeks) and a late seroconversion at range f (21 to 25 weeks) ($p < 0.05$) (Fig. 2).

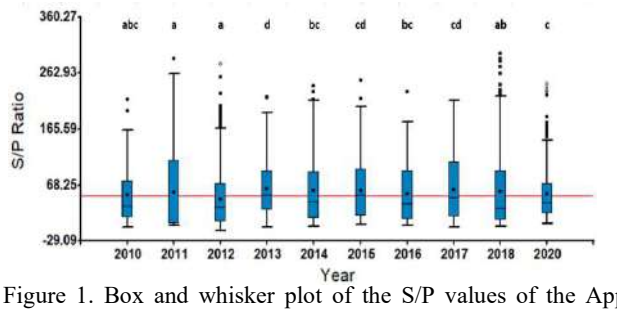

Figure 1. Box and whisker plot of the S/P values of the App serology of 18 swine farms classified by year. Red line shows threshold level (S/P ratio of 50) for a positive result. [abcd] Different letters among years show statistical significance difference ($p<0.05$).

Discussion and Conclusions

Average serological differences between years were observed ($p<0.05$), with higher values on 2011 and 2017 and lower values on 2010, 2012 and the first quarter of 2020 (Fig. 1).

The analysis of the results per year considering the ranges of ages (Fig. 2) showed that in 2010 after the decay of the passive immunity they remain negative, similar to 2011 serological results, despite the different in the level of maternal immunity showed by these years. These might be because there were few samples those years. In addition, in 2011 the maternal immunity decays later that the other years, which may be because the higher passive immunity.

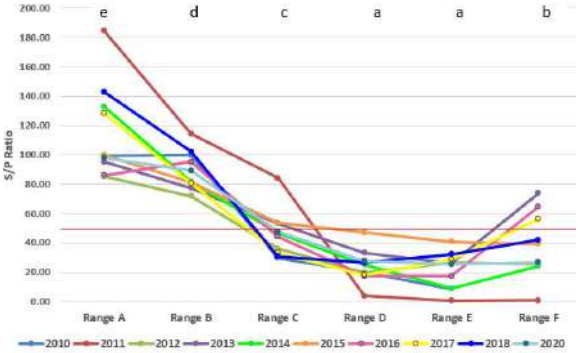

Figure 2. Line chart of the average S/P values of App serology by year, classified by age range of 18 swine farms. Red line shows threshold level (S/P ratio of 50) for a positive result. [abcd] Different letters show statistically significant differences between average age range, calculated from all the samples of all years in that range ($p<0.05$).

From 2012 to 2020 few differences were observed with a marked trend to de decay of passive immunity and a time of seroconversion through range F. Nevertheless, 2015 and 2020 although they have a lot of samples, they maintain as negative from range D to F (13 to 25 weeks).

This data suggest that the passive immunity drops around 9 to 12 weeks of age, and a late seroconversion at 21 weeks of age or more.

References

1. Sassu, E., et al. 2017. Transboun Emerg Dis, 65, 72-90.
2. Bosse, J., et al. 2018. Vet Microbiol, 217, 1-6.
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   Muñoz, D., et al. 2008. Arch Med Vet, 40, 147-15

Seroprevalence of *Actinobacillus pleuropneumoniae* in swine intensive production systems from Chile

Laura Peralta[1], Aline Vera[1], Álvaro Ruiz-Ormeño[1], Constanza Villarroel[1], Benjamín Céspedes-Cortés[1], Joaquín Álvarez-Norambuena[1], Álvaro Ruiz[1]

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Introduction

Actinobacillus pleuropneumoniae (App) is the etiologic agent of swine pleuropneumonia, a highly infectious, often fatal disease. Causing significant economic losses worldwide due to mortality and treatment costs (1). The economic losses caused for App in Chile have led to researches in order to control outbreaks in the different intensive production systems (2). There are 18 serovars and 2 biotypes of this bacteria (3). Serology is important to understand epidemiology of an App outbreak (4). Not many studies have been done in Chile about the serological behavior of App, because of that, a retrospective study of the last 10 years of serological results is presented.

Materials and Methods

App ELISA serological results samples of 18 swine production farms from Chile, carried out during the years 2010 to the first quarter of 2020, in the Swine Diagnostic Laboratory of the Universidad de Concepcion, Chile were analyzed. In this study, year 2019 was not considered due to the low number of samples.

One thousand nine hundred and seventy-six serum samples from the commercial line, of different age were studied. The samples were classified by weeks; D: 13 to 16 weeks; E: 17 to 20 weeks; F: 21 to 25 weeks. Groups from 3 to 12 weeks were not considered to avoid maternal immunity bias.

Samples were analyzed by ELISA, using the commercial IDEXX APP-ApxIV Ab Test (IDEXX Laboratories, Westbrook, ME, USA), following the standardized protocol by the manufacturer.

Statistical analysis was made by Kruskal-Wallis as the distribution was not normal.

Results

An overall seroprevalence of 22.62% was observed among the years of study. Different seropositive results by year were reported ($p < 0.05$) (Table 1). Differences by ranges were also reported ($p < 0.05$) (Table 2).

Discussion and Conclusions

In this study, significant differences were found for the seroprevalence result by year, where the highest seroprevalence observed was 2013 and 2015 (35.76% and 35.45%, respectively), without statistical difference among them. However, the lowest seroprevalence observed was on 2010 and 2011, with 5% and 0%, respectively, but these years, got few samples without statistical difference between those years ($p > 0.05$). Difference between years (Table 1) might be influenced for the existence of different serovars of App in Chile, as describes before by Muñoz et al (2008).

Table 1. Percentage of positive, suspect and negative ELISA results of swine samples between 2010 to 2020, classify by years of sampling.

Year	Pos %	Susp %	Neg %
2010[abc]	5.00%	0.00%	95.00%
2011[a]	0.00%	0.00%	100.00%
2012[a]	17.98%	7.36%	74.66%
2013[d]	35.76%	6.67%	57.58%
2014[bc]	8.81%	8.18%	83.02%
2015[cd]	35.45%	7.79%	56.76%
2016[bc]	21.37%	8.40%	70.23%
2017[cd]	29.84%	4.84%	65.32%
2018[ab]	17.78%	3.70%	78.52%
2020[c]	9.91%	9.91%	80.19%
Total	22.62%	6.93%	70.45%

[abcd] Different letters among years show statistical significance differences ($p<0.05$).

When seroprevalence averages from 2010 to 2020 by age range were compared, statistical difference was observed in all ranges ($p < 0.05$). But the higher seroprevalence reported was in range F, that might be due to the late seroconversion.

Table 2. Percentage of positive, suspect, and negative ELISA results of swine samples between 2010 to 2020, classify by age range.

Age	Pos %	Susp %	Neg %
Range D[b]	21.35%	6.92%	71.73%
Range E[a]	17.38%	6.98%	75.63%
Range F[c]	29.47%	6.90%	63.64%
Total	22.62%	6.93%	70.45%

[abc] Different letters among age ranges show statistical significance differences ($p<0.05$).

From the overall results, 88 % of the swine intensive production farms were positive to the presence of App. Without regard to the passive immunity the overall seroprevalence was 22.62%.

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1. Sassu, E., et al. 2017. Transboun Emerg Dis, 65, 72-90.
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4. Teshima, K., et al. 2017. J Vet Med Sci, 17-0374.

Fecal microbiota transplantation (FMT) from healthy piglets affects healthiness gain and bacterial diversity in runt piglets

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Introduction

Fecal Microbiota Transplantation (FMT), also known as a stool transplant, is the process of transplantation of fecal bacteria from a healthy individual into a diseased recipient (1). Its effectiveness has been used in clinical trials for the treatment of Clostridium difficile infection of human cases. Mortality and morbidity of new born piglets continues to be a major economic burden for the swine production (2). The greatest incidence of death occurs within the first 3–4 days following birth, especially among neonates of low birth weight ("runts") or decreased vitality. The runt pig may be more susceptible to metabolic or physiological stressors than the normal sized littermate. The aim was to investigate the potential utility of FMT to promote health and higher fecal microbiome diversity in runt pigs.

Materials and Methods

On the weaning day, twenty pigs (15 runt pigs and 5 healthy pigs) were randomly assigned to 4 groups: treated with fecal microbiota transplantation (FMT) and fed with 0.8% galactooligosaccharide (GOS). Serum and fecal samples (week 3, 5, and 7) were collected and examined antibody titers of FMDV and fecal bacterial communities by next generation sequencing (iSeq100). Average daily weight gain (ADWG) were evaluated.

Results

Over the 4-week FMT treatment period, the FMT group showed higher diversity of bacterial communities, along with increased FMDV antibody levels and ADVG. FMT treated pigs showed a positive correlation from healthy pigs in FMDV antibody titers, alpha and beta diversity, and ADWG (for all: $p<0.05$). From out PcoA analysis of beta diversity, the gut microbiomes of fecal transplant runt pigs clearly shifted toward the communities of the healthy pigs.

Conclusions and Discussion

The FMT could be considered as one of medical treatments as a whole. When administered especially with probiotics, the overall health condition of the runt pigs was promoted, giving them higher fecal microbiome diversity. Further research is remained to understand the mechanism behind this relationship between health promotion of rubbish pigs and the FMT: how the large-scale microbiome modulation could be adapted to increase the health of runt pigs.

Acknowledgments

This research was supported by Technology Development Program (Project No. 1116043-1) for Bio-industry, Ministry for Agriculture, Food and Rural Affairs, Republic of Korea.

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2. Palmer R. 2011. *Nature Med* 17:150–152.

Application of propidium monoazide (PMA)-based real-time qPCR for detection of viable *Lawsonia intracellularis* as a clinical post-antibiotic susceptibility method from feces of porcine proliferative enteropathy cases

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Introduction

Lawsonia intracellularis (LI) is an intracellular entero-pathogen causing two main clinical manifestations in pigs: an acute hemorrhagic form often called proliferative hemorrhagic enteropathy (PHE), and a more chronic proliferative form often referred as proliferative intestinal adenomatosis (PIA) (1). Due to extreme difficulty of *in vitro* culture of LI it is difficult to analyze the effects after antibiotics through bacterial culture.

In the treatment of ileitis, there are no laboratory tests that can verify the efficacy of treatment after antibiotic treatment. Because it is not easy to test the bacteria discharged into the feces, and simple real-time PCR cannot distinguish between living and dead bacteria. Propidium monoazide (PMA) is a photoreactive DNA-binding dye that inhibits PCR amplification by modifying chromosomal DNA. PMA is intercalated into the dead bacteria and covalently cross-linked to bacterial DNA, which strongly inhibits amplification (2).

Therefore, the objective of the present study was to determine the application of PMA-based real-time qPCR for detection of viable *Lawsonia intracellularis* as a clinical post-antibiotic susceptibility method from feces of porcine proliferative enteropathy cases

Materials and Methods

LI were passaged and harvested from infected IEC-18 cells. Dead LI were prepared by exposing live LI to 70% isopropyl alcohol for 30 min. Seven samples with dead:live ratios of 0:100 (live control), 10:90, 30:70, 50:50, 70:30, 90:10, and 100:0 (dead control) were prepared for real-time qPCR and cell culture. Fecal samples from 3 pig farms infected with LI were used in this study. PMA-treated and conventional real-time qPCR were performed on feces before and 10 days after antibiotic administration (tiamilin hydrogen fumarate [4kg/ton]).

Results

As a result, PMA-treated real-time qPCR was useful for determining LI viability. In the pig farms samples treated with antibiotic administration were presented improved clinical signs and the median deltaCT value of PMA-treated or non-PMA treated were 5.8 and 1.7, respectively, whereas that in other pig farm with clinical signs were 1.5 and 1.1. It is postulated that pig farm with improved clinical signs contained more dead LI than those of clinical LI infection cases.

Conclusions and Discussion

PMA real-time qPCR is a useful approach for differentiating dead from live LI in fecal samples and for detection of viable *Lawsonia intracellularis* as a clinical post-antibiotic susceptibility method from feces of porcine proliferative enteropathy cases

Acknowledgments

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Fecal microbiota transplantation (FMT) from healthy piglets improves health condition and microbial diversity in runt piglets

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Introduction

Fecal Microbiota Transplantation (FMT), also known as a stool transplant, is a process of transplantation of fecal bacteria from healthy donors into a diseased recipient (1). Its effectiveness has been used in clinical trials as one of treatment options toward Clostridium difficile infection of human cases. Mortality and morbidity of new born piglets continues to be a major economic burden in swine industry (2). The greatest incidence of death occurs within the first 3–4 days after birth, especially among neonates of low birth weight ("runts") or decreased vitality. The runt pig may be more susceptible to metabolic or physiological stressors than the normal sized littermate. The aim of this study was to investigate the potential utility of the FMT to promote the health and higher fecal microbial diversity in runt pigs.

Materials and Methods

On the weaning day, twenty pigs (15 runt pigs and 5 healthy pigs) were assigned to 4 groups: treated with fecal microbiota transplantation (FMT) and fed with 0.8% galactooligosaccharide (GOS). Serum and fecal samples (week 3, 5, and 7) were collected and examined antibody titers to FMD which is the mandatory measure in Korea and fecal microbial communities by next generation sequencing (iSeq100). Average daily weight gain (ADWG) were evaluated.

Results

Over the 4-week FMT treatment period, the FMT group showed higher diversity of bacterial communities, along with an increased antibody levels to FMDV, and ADWG. Those FMT treated pigs showed a positive correlation with healthy pigs in FMDV antibody titers, the alpha and beta diversities, and ADWG (for all: $p<0.05$). From the PcoA analysis of the beta diversity, the gut microbiomes of runt pigs administered with FMT clearly shifted toward the communities of those of the healthy pigs.

Conclusions and Discussion

The FMT could be considered as one of medical treatments as a whole. When administered especially with pro- and pre-biotics, the overall health condition of the runt pigs was highly promoted, giving them higher fecal microbiome diversity. Further research is remained to understand the mechanism behind this relationship between health promotion of rubbish pigs and the FMT: how the large-scale microbiome modulation could be adapted to increase the health of runt pigs.

Acknowledgments

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Application of propidium monoazide (PMA)-based real-time qPCR as a clinical post-antibiotic susceptibility test to detect viable *Lawsonia intracellularis* from fecal samples of porcine proliferative enteropathy cases

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Introduction

Lawsonia intracellularis (LI), so-called ileitis pathogen in field, is an intracellular entero-pathogen causing two main clinical manifestations in pigs: an acute hemorrhagic form often called proliferative hemorrhagic enteropathy (PHE), and a more chronic proliferative form often referred as proliferative intestinal adenomatosis (PIA) (1). Due to extreme difficulty of *in vitro* culture of LI it is difficult to analyze the effects of antibiotics as well through bacterial culture.

In the treatment of ileitis, there are no laboratory tests that can verify the efficacy of antibiotics after treatment. Because it is not easy to test the bacteria shed through feces, and the simple real-time PCR cannot distinguish living bacteria from the dead. Propidium monoazide (PMA) is a photoreactive DNA-binding dye that inhibits PCR amplification by modifying chromosomal DNA. The PMA is intercalated into the dead bacteria and covalently cross-linked to bacterial DNA, which strongly inhibits amplification (2).

Therefore, the objective of the present study was to determine whether the application of the PMA-based real-time qPCR is possible for the detection of the viable *Lawsonia intracellularis* as a clinical post-antibiotic susceptibility test from feces of porcine proliferative enteropathy cases

Materials and Methods

LI were passaged and harvested from infected IEC-18 cells. Dead LI were prepared by exposing live LI to 70% isopropyl alcohol for 30 min. Seven samples with dead:live ratios of 0:100 (live control), 10:90, 30:70, 50:50, 70:30, 90:10, and 100:0 (dead control) were prepared for real-time qPCR and cell culture. Fecal samples from 3 pig farms infected with LI were used in this study. PMA-treated and conventional real-time qPCR were performed on fecal samples before and 10 days after administration of antibiotics (tiamilin hydrogen fumarate [4kg/ton]).

Results

As a result, the PMA-treated real-time qPCR was useful for determining LI viability. In the pig farms, samples from pigs which treated with antibiotics and improved clinical signs, presented the median ΔCT value of PMA-treated or non-PMA treated as 5.8 and 1.7, respectively, whereas samples from pigs which non-treated with antibiotics and clinical signs were 1.5 and 1.1. It is postulated that pig farm with improved clinical signs contained more dead LI than those of clinical LI infection cases.

Conclusions and Discussion

The PMA real-time qPCR is a useful approach for differentiating dead LI from live one in fecal samples and for detection of viable LI as a clinical post-antibiotic susceptibility test from fecal samples of porcine proliferative enteropathy cases

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Acknowledgments

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Characteristics virulence factor combination of verotoxin 2e producing *Escherichia coli* isolated in Korean pig farms

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Introduction

Porcine Edema disease (ED) is caused by verotoxin-producing *Escherichia coli* (STEC) strains which produce verotoxin 2e (VT2e) and may possess F18 fimbrial unit. Following intestinal colonization of STEC, VT2e enters the systemic circulation and causes vascular necrosis of arterioles in the brain and gastrointestinal tract. The clinical severity of ED varies depending on the farm, as some affected farms show typical clinical signs, whereas others show subclinical symptoms. Although STEC strains producing typical ED clinical signs are known to express identical VT2e regardless of serotypes (1), the genomic characteristics of VT2e by different clinical manifestations are not yet identified. In this study, Korean STEC strains were isolated from pigs with classical or subclinical ED (SED), and the full VT2e nucleotide sequence of each strain was analyzed. Also other genes such as EAST1, STs, LT and adhesions to determine characteristics of various combination of the VT2e containing E.coli.

Materials and Methods

A total of 57 diarrheic samples of 40 to 100 days-old pigs were collected from 9 conventional pig farms in different regions of South Korea. Eleven were from piglets showing the typical ED clinical signs (sudden death/palpebral edema/ neurological signs) and 46 were from unthrifty piglets. The samples were individually streaked on a MacConkey agar, incubated overnight, and five susceptible colonies in each plate were tested for 13 virulence factors (toxins, LT/STa/STb/VT2e/EAST1; adhesins, F4/F5/F6/F18/F41/paa/AIDA-I/EAE) by polymerase chain reaction. The virulence gene combination was determined, and the entire VT2e domain of STEC strains was amplified by using gene-specific primers. The individual amplicons were sequenced by the Sanger's method, and the amino acid sequence identities were estimated in BioEdit 7.2.5.

Results

The detection rate of VT2e gene was 37% (21/57), and STEC strains were classified into 3 groups (Table 1): **1)** STEC without fimbriae (13/21), **2)** STEC producing VT2e and F18 (6/21), **3)** STEC producing enterotoxins, VT2e and F18 (2/21). While the STEC strains isolated from pigs with typical ED clinical signs all belonged to Group 2, the strains isolated from pigs with subclinical ED belonged to Group 1 or 3. Interestingly, all STEC strains shared 100% of amino acid sequence identity of VT2e regardless of groups and virulence factor combinations (Table 2).

Table1. Virulence factor combination of Korean verotoxin-producing *Escherichia coli* strains.

Group	Toxins	Adhesins	Clinical manifestations
1 (n=13)	VT2e VT2e:STb	- -	SED
2 (n=6)	VT2e VT2e	F18:AIDA F18:AIDA:paa	ED
3 (n=2)	VT2e:LT:STa VT2e:STa:STb :EAST1	F18 F18:EAE:paa	SED

ED, clinical edema disease; SED, subclinical edema disease.

Table2. Pairwise amino acid sequence comparison between verotoxin 2e domain of Korean verotoxin-producing *Escherichia coli* strains.

	Sequence identity (%) of VT2e domain between STEC groups		
	Group 1	Group 2	Group 3
Group 1	ID	100.0	100.0
Group 2		ID	100.0
Group 3			ID

VT2e, verotoxin 2e; STEC, verotoxin-producing *Escherichia coli*; ID, identical

Conclusions and Discussion

Porcine ED is widespread in the swine industry worldwide, presenting diverse clinical manifestations depending on the affected pig farm. In Korea, VT2e has been frequently (37%) detected. All the STECs causing typical ED clinical signs presented only VT2e without enterotoxins, F18 and AIDA, while the strains causing subclinical ED (diarrheic/wasting) showed various virulence gene profiles; mainly VT2e without adhesins (13/15). In view of our results, the severity of ED does not correlate to the genomic characteristics of VT2e since all STEC strains possess identical VT2e amino acid sequences, but regarding our results may be influenced by other virulence factors The establishment of risk assessment criteria for the clinical severity of ED by farm levels is necessary for prevention of acute ED outbreaks, and the classification system for STEC strains seem to be a potential index for the risk assessment.

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Survey of *Mycoplasma hyopneumoniae* clinical symptoms and vaccination in sows

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Introduction

Mycoplasma hyopneumoniae (Mhp) is the main pathogen of Enzootic Pneumoniae (EP) in pigs, an economical relevant infection in major pig producing countries. When looking into textbooks (1,2), it can infect pigs at all ages but clinical symptoms are mainly seen in growing pigs. Adult animals are supposedly rarely clinically affected except for acute outbreaks in naïve farms. However, detailed description of clinical symptoms in adult pigs is limited. Therefore, Boehringer Ingelheim developed a survey together with Pig333 to get data of clinical symptoms and vaccination strategies against Mhp in sows.

Material and Methods

In total, 566 responses with 493 coming from veterinary practitioners/consultants were included in the evaluation. Responses came from all parts of the world (Table 1).

Region	% Participants (n)
Africa (Af)	0,89 (5)
Asia/Oceania (As-O)	15,43 (87)
Europe (EU)	50,18 (283)
North America (NA)	14,36 (81)
South America (SA)	19,15 (108)
Total	100,00 (564)

Results

The most interesting findings were:

- 46,3% of respondents have experienced one or more Mhp outbreaks in sow farms.
- In most areas, the majority of outbreaks (31 to 40%) has been observed in Mhp positive farms with piglets but no sow vaccination – only in Asia the highest observation of outbreaks was in negative farms without sow or piglet vaccination.
- Clinical symptoms were reported to be **cough in suckling piglets** (36,0% of answers), **cough in sows** (27,5%), **fever in sows** (12,5%), **weak-born piglets/ increased pre-weaning mortality** (12,2% and **abortions** (6,1%).
- In farms that vaccinate sows, a vaccination schedule of every 6 month is independently of the region the mostly reported schedule (25,0 to 28,4%) followed by pre-farrowing vaccination (19,2 to 32,6%).

Discussion

Mhp outbreaks in sow farms are rarely mentioned in Textbooks and not described at all in scientific papers. However, from this survey Mhp outbreaks in sows are obviously not unusual. Unexpectedly, outbreaks were mainly reported to occur in Mhp positive farms with piglet vaccination only. Mhp vaccination of only piglets may be due to the missing vaccine label claim for sows in some regions and countries (e.g. Europe) where respondents reported also the highest rate of herds that do not vaccinate sows (30% compared to 17.3% in NA).

Sow vaccination on a regular basis to control Mhp in farms may not be perceived beneficial in all regions, even though there are some papers describing more severe respiratory symptoms in farms with Mhp instable sows (3, 4). In addition there are papers demonstrating that sow vaccination can reduce Mhp transmission from sows to piglets (5, 6) and in addition to piglet vaccination can reduce lung lesions in pigs from vaccinated sows at slaughter (5, 7).

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Field testing of a new needle-free, intramuscular injection device for pigs

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Introduction

Usually injections and vaccinations in pigs are applied with syringes and hypodermic needles. Even though this method has been established for decades, there are a few negative aspects with regard to needles like broken needle tips in meat (consumer safety), needle pricks (worker safety) as well as pathogen transmission by needles and use of blunt needles (animal welfare)[1-3].

Some vaccine manufacturers have developed needle-free intradermal vaccine application, which overcome most of the downsides of injections with needles. However, due to the low volume of intradermal vaccines (0.2 ml/dose) new registrations are needed for each vaccine and there is no simple alternative for administering the vaccine in case of device malfunction during vaccination. To overcome the downsides of both needles as well as intradermal application, Henke-Sass Wolf, together with Boehringer Ingelheim Vetmedica have developed a new autonomous hand-held device (FreVAX™) for needle-free intramuscular application.

Laboratory studies and in vitro studies have been carried out previously to demonstrate PRRS virus survival with regard to the pressure and intramuscular delivery of vaccines with low viscosity in 3-5 week old pigs (unpublished). The aim of this study was to evaluate the degree of PRRS viremia and serological response after vaccination in a field setting.

Materials and Methods

In total, 110 pigs of one farrowing batch from a PRRS negative herd at the age of about 3 weeks were used on arrival in a nursery-finishing farm in Italy. Pigs were randomly divided into 2 groups: G1 (n=55) was vaccinated with 2 ml of a Modified Live PRRS vaccine (Ingelvac® PRRSFLEX EU, Boehringer Ingelheim Vetmedica GmbH) with the needle-free device and G2 (n=55) was vaccinated with the same vaccine but with a regular needle on the day of arrival (study day 0; SD0). At SD0, 20 piglets were bled to confirm PRRS negative (antibody and virus) status (IDEXX ELISA and PRRS qPCR). On SD6 and SD31, blood samples from all animals were tested for PRRS viremia and PRRS serological response (PRRS qPCR and IDEXX ELISA). Data were compared using a non-inferiority test or a T-test and in case of non-normal distribution, the Wilcoxon rank-sum test.

Results

The pre-test at SD0 confirmed the PRRS negative status of the pigs prior to vaccination. ELISA and PCR study results are summarized in Table 1 and 2, respectively. As expected, serology was still negative in all animals at SD6. Vaccination with the needle-free device was not significantly different and non-inferior compared to the needle application except for the percentage of animals that were viremic on SD31 post vaccination (Table 2). However, a higher percentage of animals were seropositive on SD31 and viremia started earlier in needle free vaccinated pigs.

Table 1. Serology (ELISA S/P values) of piglets vaccinated with the Modified Live PRRS vaccine

	ELISA SD6		ELISA SD31	
	FreVAX	Needle	FreVAX	Needle
Mean S/P ratio	0,00 [d]	0,00	1,28[c]	1,14
% pos. samples	0,00	0,00	96,3[a]	85,45

[a]test for non-inferiority passed; [b] test for non-inferiority not passed; [c] t-test; no significant difference (p>0,05); [d] Wilcoxon test; no significant difference (p>0,05)

Table 2. Viremia (cT value) of piglets vaccinated with a Modified Live PRRS vaccine

	PCR SD6		PCR SD31	
	FreVAX	Needle	FreVAX	Needle
Mean cT	25,71[d]	22,85	16,23[d]	18,62
% pos. samples	85,45[a]	74,55	47,17 [b]	58,18

[a] test for non-inferiority passed; [b] test for non-inferiority not passed; [c] t-test; no significant difference (p>0,05); [d] Wilcoxon test; no significant difference (p>0,05)

Conclusions and Discussion

There was no indication that a MLV PRRS vaccination with the needle free device was inferior to regular needle application based on seroconversion. The higher seroconversion rate with slightly higher S/P ratios in the needle free device group on SD31 may even be an indication of an earlier immune response.

At SD6, 11% more animals were viremic (as determined by PCR) in the needle free group, which may be the reason for the slightly earlier serological response. The only statistically significant difference was the inferiority with regard to the frequency of viremic animals detected on SD31, which may be the consequence of earlier viremia, earlier immune response and therefore earlier clearance of the vaccine virus.

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Dynamics of *Mycoplasma hyopneumoniae* genotypes from outbreaks of enzootic pneumonia in fattening pigs

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Introduction

Mycoplasma hyopneumoniae (*M. hyopneumoniae*) still causes significant economic losses in the pig industry. The genetic variability among geographic area and within a farm is know but no informations are avilable regards to how an outbreak evolves over time. Aim of this study were to describe the variability of *M. hyopneumoniae* genotypes during acute and recovering phase of enzootic pneumonia outbreaks.

Materials and Methods

Ten fattening farms with a history of *M. hyopneumoniae* were chosen for this study. During an outbreak of enzootic pneumonia, 30 pigs per farm were randomly selected and identified. For each animal were collected tracheobronchial swabs in acute and recovery phase (30 days later), named as T1 and T2 respectively. The samples were analyzed by real-time PCR and the positive ones were after genotyped by Multiple Locus Variable number tandem repeat Analysis (MLVA). Infection with one *M. hyopneumoniae* genotype were defined as single (SN) while infection with more than one genotype were defined as mixed (MX).

Results

The PCR results showed similar prevalence of *M. hyopneumoniae* in T1 and T2 with not statistical difference. Genotyping reported 9.6% and 15.2% of MX infection in T1 and T2 respectively. In five farms (A, B, C, E and G) rate of pigs with MX infections increase from T1 to T2 (Figure 1).

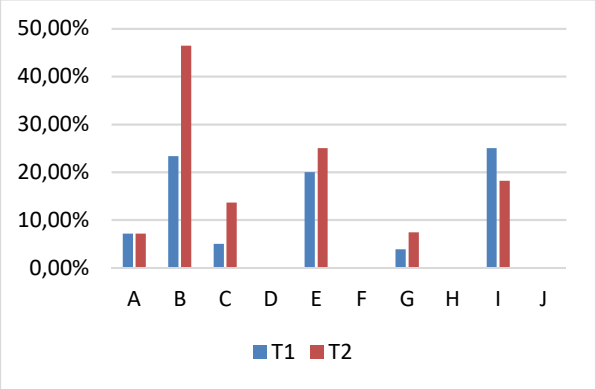

Figure 1. Rate of MX infections in acute (T1) and recovery phase (T2).

In the farms in which we detect only SN infection at T1 (D, F, H and J) pigs continue to have SN infection (Figure 1) in T2.

Conclusions and Discussion

Results suggest that prevalence of *M. hyopneumoniae* does not increase quickly in the herd during an outbreak, confirming data already reported in literature. MX infections when present in a farm, increase during an outbreak of enzootic pneumonia and involves more pigs. Conversely, farms with SN infection do not undergo the entry of multiple genotypes but tend to remain SN over time. These findings lead us to suppose that *M. hyopneumoniae* is conserved within each farm and genotypes also. Furthermore, during an outbreak the farms with multiple genotypes show a spread of different genotypes within a herd, with an increasing of MX infections pigs. A deeper comprehension of this dynamics would need longer longitudinal study over time.

Acknowledgments

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Study comparing Aivlosin® WSG with Tylan® to Control *Lawsonia* in Nursery/Finishing Swine in Denmark

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Introduction

This study compared treatment with the novel water soluble macrolide Aivlosin® and water soluble Tylan® in a pig unit in Denmark with a history of *Lawsonia intracellularis* and respiratory problems in grower/finisher pigs. Pigs were positive for *Mycoplasma hyopneumoniae* and given a single vaccination at weaning.

Materials and Methods

The trial was conducted in a wean to finish unit based in Skanderborg, Denmark into which 700 weaned pigs entered every 2 weeks.

The trial was performed on two consecutive batches of 350 pigs. On arrival at the nursery, 350 pigs were allocated randomly to one of two treatment groups and individually weighed. Treatment of both test groups with their respective medication began when 10% of the pigs showed signs of scouring; both treatments started on the same day and were given in drinking water. Aivlosin® 625 mg/g Granules for Use in Drinking Water for Pigs (ECO Animal Health) was given at a dose rate of 5.0 mg tylvalosin/kg bodyweight daily for five consecutive days and Tylan® Soluble Powder (Elanco) was given at 8 mg tylosin/kg bodyweight daily for 5 consecutive days. Medicated water was made up fresh on a daily basis for both products.

Parameters measured for each group were mortality, weekly and overall average faecal scores (from one week after placement in the finishing area until 1 week prior to slaughter), bodyweights at each weighing point, average daily gain in finishing, feed conversion ratio (FCR) and lung scores on a subsample of 35 pigs per treatment and per batch (Goodwin method). Statistical analysis was performed in SAS version 9.4. Data normally distributed were analyzed using multivariate linear model. Mortality was analyzed using Kaplan-Meier plots and proportional hazards models.

Results

Results are shown in the table below.

	Aivlosin®	Tylan®
Av. Faecal Score (p-value 0.51)	0.4	0.41
Pigs requiring additional treatment (%) (p-value 0.02)	8.6[a]	13.9[b]
Pigs requiring additional treatment for ileitis (%) (p-value 0.03)	6.6[a]	11.0[b]
Mortality (%) (p-value 0.05)	1.9[a]	4.9[b]
FCR	2.16	2.24
ADG (kgs/day) (p-value 0.19)	0.990	0.888
Pneumonic lungs at slaughter (%) (p-value <0.01)	19[a]	39[b]
Pneumonia Index (IPP) (p-value 0.07)	0.85	2.01

°Superscripts indicate statistically significant differences.

Conclusions and Discussion

In this study, pigs in the Aivlosin® group had significantly lower percentage of pigs needing additional treatments, lower mortality and lower percentage of pneumonic lungs than pigs in the Tylan® group. Pigs treated with Aivlosin® received 60% less antibiotic on a mg/kg basis than did the pigs treated with Tylan®, excluding additional treatments.

Field study comparing Aivlosin® WSG with Denagard® in Brazilian pigs naturally infected with *Mycoplasma hyopneumoniae* and secondary respiratory pathogens.

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Introduction

This study compared treatment with Aivlosin® WSG (Aivlosin® 625 mg/g Granules for Use in Drinking Water for Pigs, ECO Animal Health Ltd.) and Denagard® 45% (Elanco) in a pig unit in Brazil with respiratory problems in grower/finisher pigs. Pigs were positive for *Mycoplasma hyopneumoniae* and secondary respiratory pathogens including *Pasteurella multocida*, *Haemophilus parasuis* and *Actinobacillus pleuropneuminae*.

Material and methods

1,200 weaned pigs (22 days of age) were allocated randomly to one of two treatment groups:

T1 – Aivlosin® WSG at 5 mg tylvalosin/kg BW/day
T2 –Denagard® 45% at 15 mg tiamulin/kg BW/day
Both treatments were given in drinking water at 30-34, 65-69 and 120-124 days of age.

Health and performance parameters were measured. Pigs were weighed individually at weaning, day 58 and day 152. Coughing scores, percentage mortality, percentage pigs sent to the infirmary, Weight Gain, Average Daily Gain and Feed Conversion Ratio (FCR) were measured over these periods. Lung scores, using the IPP (Index for Pneumonia) Embrapa method (Piffer and Brito, 1991) for prevalence of pneumonic lungs and pleurisy assessments were performed on 240 pigs randomly chosen at slaughter, 120 per medication group. Average lung lesion scores were measured using the Goodwin method (Goodwin //, 1973)

Results

Results are shown in Table 1. Aivlosin®-treated pigs had a significantly lower coughing incidence in the finishing period compared to Denagard®-treated pigs.

Table 1. Health and Performance Parameters (Embrapa method).

	Aivlosin®	Denagard®
Coughing Score - Finishing (p<1%)	0.9%	1.5%
Pneumonic Lungs (NS)	49.2%	56.7%
Lungs with Pleurisy (NS)	6.7%	5.8%
Lungs with Abscesses (NS)	0.0%	1.7%
Feed Conversion Ratio - Nursery (NS)	1.53	1.53
Feed Conversion Ratio - Finishing (p<5%)	1.90	1.94
Feed Conversion Ratio - Total (p<5%)	2.02	2.05

NS indicates no statistically significant difference

Average lung lesion scores under the Goodwin method for Denagard® were 8.51 and for Aivlosin® were 9.83; the difference not being significant. FCR was significantly lower (P<5%) in Aivlosin®-treated pigs compared to Denagard®-treated pigs in the finishing period and over the total period. Prevalence of pneumonic lungs, associated with mycoplasmal pneumonia, at slaughter was lower for Aivlosin®-treated pigs (49.2%) than Denagard®-treated pigs (59.7%), though the difference was not statistically significant.

Conclusion

In this study, both groups performed well. Aivlosin®-treated pigs had significantly lower coughing incidence, lower FCR in the finishing and total periods and lower prevalence of lungs with lesions typical of mycoplasmal pneumonia while using only 33% of the amount of antibiotic used by Denagard®-treated pigs.

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Achieving the impossible: early detection of *Mycoplasma hyopneumoniae*

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Introduction

The cost of *Mycoplasma hyopneumoniae* (MHP) ($375-400 million annually) has pushed the industry towards elimination, but the slow transmission, complexity of swine production, and the absence of optimized surveillance has hindered the development of effective control/elimination programs. Therefore, the purpose of this study was to compare the MHP probability of detection (PD) using tracheal swab (TS), serum (S), and oral fluid (OF) samples.

Materials and Methods

This study was conducted in one room (46 pens, ~28 pigs per pen, ~1250 pigs) of a wean-to-finish site sourced with 21-day-old MHP-negative barrows. At 9 weeks of age, 10 seeder pigs in a centrally located pen were inoculated with MHP lung homogenate, with the remainder of the pigs in the pen deemed contact pigs (n=18). At the time of inoculation and every two weeks thereafter (7 total samplings), one OF, one TS, and 4 S samples were collected from each of the 45 contact pens. A total of 315 TS, 1260 S and 315 OF were collected. For individual animal samples (TS, S), pigs were selected at random at each sampling. OF were collected at the pen level. Samples were tested for DNA (TS and OF) or antibody (S). Using the field data, a hierarchical Bayesian model based on a latent spatial piecewise exponential model[1] was developed to estimate the MHP spread as well as sensitivities of three sample and test types. A total of 10,000 simulations were performed to ensure a robust PD estimate by sample size, type and prevalence. The MHP transmission rate was determined from the field data. With this, 2 scenarios were modeled to estimate the effect of time and initial prevalence on PD. The infection began with one or five positive pig(s) in one pen and PD estimates were calculated by sample size, prevalence, and days post infection (DPI) until 100% PD was achieved for each sample type and size.

Results

All seeder and contact pigs were positive by TS and S by 7 and 28 DPI, respectively. For contact pens, the first positive occurred at 14 DPI by S, 28 DPI by TS and 42 DPI by OF. At ~100 DPI, all TS and S were positive. Table 1 shows the PD estimates for Scenarios 1 & 2 for sample sizes 30 and 90 samples.

Conclusions and Discussion

The results of this study confirmed previous knowledge on the transmission and spread of MHP within swine populations. Due to the nature of MHP, surveillance remains challenging for the swine industry. Prior to this study, PD estimates for available diagnostic tests were unavailable.

PD estimates showed that TS provided the earliest detection of MHP, especially at large numbers. For example, if the initial infection starts with 5 pigs, 90 TS will provide a PD of 0.58 at 7 DPI. However, TS are challenging to collect, involve more pig handling, and are 5 times more expensive than S testing. The first detection in S, at a PD of >0.5, occurred at 42 DPI. While S remains a robust, economical and a practical sample to collect, the delayed immune response limits its use for early detection. For both scenarios and sample sizes, OF provided a PD ≥ 0.5 at 42 DPI. PD estimates for previous timepoints were significantly lower compared to TS and S. These data show that OF are not an appropriate sample for early detection of MHP. However, routine collection of OF in known positive populations (MHP present >42 days) may provide an economical and welfare-friendly solution for monitoring MHP.

The results obtained in this study will significantly improve surveillance for this elusive pathogen.

Table 1. PD estimates for Scenario 1 & 2

Sample size	# of initially infected pigs	Sample Type	Days post infection				
			0	7	21	42	56
30	1	TS	0.03	0.16	0.40	0.66	0.85
		S	0.00	0.00	0.02	0.37	0.62
		OF	0.00	0.00	0.00	0.50	0.69
	5	TS	0.11	0.25	0.52	0.79	0.94
		S	0.00	0.00	0.10	0.45	0.71
		OF	0.00	0.00	0.00	0.53	0.75
90	1	TS	0.07	0.38	0.72	0.91	0.97
		S	0.00	0.00	0.06	0.72	0.90
		OF	0.00	0.00	0.00	0.85	0.95
	5	TS	0.31	0.58	0.84	0.97	0.99
		S	0.00	0.00	0.26	0.81	0.96
		OF	0.00	0.00	0.00	0.87	0.97

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Efficacy of vaccination against *Lawsonia intracellularis* in a farrow-to-finish farm operating continuous flow in Japan

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Introduction

The intestinal bacterium, *Lawsonia intracellularis* (*Li*) is the causative agent of porcine proliferative enteropathy (PPE), which is considered to be an economically important disease especially in grower-finisher pigs. There are three forms of presentation of *Li* infection in pigs. Acute form is characterised by haemorrhagic diarrhoea and sudden death, usually seen in finishers and gilts at 4-12 months old. Chronic form is commonly observed during the grower phase, and causes diarrhoea and reduced growth rate. No obvious diarrhoea is seen in subclinical form, but it leads to growth retardation.

A commercial vaccine, Enterisol® Ileitis (Boehringer Ingelheim) has been available since 2010 in Japan, and it has been becoming more widely used in recent years. As it is an oral live attenuated vaccine, it is usually administered to pigs individually using drench. However, the vaccine efficacy was also demonstrated when it is administered to whole groups of pigs via drinking water using proportioners at a large-scale farm[1]. Good hygiene management such as cleaning and disinfection, and all-in/all-out pig flow is important to maximize vaccine efficacy. This report summarises our evaluation of the vaccine efficacy when used in a farrow-to-finish farm with 200 sows, operating continuous flow in Japan.

Materials and methods

The study was conducted in a 200-sow farm operating one-site production system, where breeding females were a crossbreed of Large White and Landrace, and breeding boars were Duroc. The farm was managed under a three-week batch system, and all-in/all-out by rooms was exercised from farrowing to nursery, but the fattening unit remained continuous production. The farm was negative for porcine reproductive and respiratory syndrome virus, but positive for *Actinobacillus pleuropneumoniae*, *Mycoplasma hyopneumoniae* and *Li*. Sporadic diarrhoea was observed in grower-finisher pigs all through the year, which resulted in variation in pig size, although the diarrhea was treated with tylosin.

Vaccination with Enterisol® Ileitis was implemented in June 2017. Two mL of the vaccine was administered to pigs one by one using drench at 3 weeks of age. Average daily gain (birth to slaughter), feed conversion ratio (whole herd and weaners-finishers) was calculated according to Yamane *et al* [2] and Ishizeki *et al* [3], and compared between before (2016) and after (2017 and 2018) vaccination.

Results

As shown in Table 1, ADG was 663 g in 2016 before vaccination, whereas it was substantially increased to 712 g in 2017 and 703 g in 2018 after vaccination. Similarly, feed conversion ratio was improved after vaccination; 3.21, 2.91, 3.01 for whole herd, 2.68, 2.47, 2.46 for weaners to finishers in 2016, 2017, and 2018 respectively.

Table 1. Results of farm performance before and after vaccination

	2016	2017	2018
ADG (g/day)	663	712	703
Feed conversion ratio (whole herd)	3.21	2.91	3.01
Feed conversion ratio (weaners-finishers)	2.68	2.47	2.46

Discussion

After implementing vaccination against *Li*, diarrhoeic symptoms and growth retardation were clearly reduced, and ADG and Feed conversion ratios were both considerably improved. The efficacy of the vaccine was demonstrated in the farm operating continuous flow for finishers.

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Eradication of tiamulin resistant swine dysentery in a multisite production system

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Introduction

Up to 2016 tiamulin resistance in *Brachyspira hyodysenteriae* (*Bhyo*), the causative agent of swine dysentery (SD), had not been diagnosed in Sweden [1]. In February 2016 tiamulin-susceptible *Bhyo* was isolated in a 500-sow herd. The herd was instantly sanitized using tiamulin, but SD was again diagnosed in June 2016. At this time, the *Bhyo* was resistant to tiamulin (Table 1).

The sow herd was partially integrated, but also sold growers to 5 fattening herds. All 5 fattening herds purchasing growers from the sow herd were contaminated as well as a 6th fattening herd through transport contacts.

Luckily, *Bhyo* was still susceptible to tylosin and tylvalosin (Table 1). However, *Bhyo* is known to rapidly develop resistance to these substances [2]. Therefore, it was decided to sanitize the sow herd using high doses of tylvalosin.

Materials and Methods

The sow herd had 7 groups of sows that farrowed every third week in an age segregated system. As the sanitation program was designed, new hygiene zones and biosecurity measures were constructed, and new hygienic measures were specified.

The sow herd was transformed into a specialised piglet producer with 1 mating unit, 1 unit for dry sows that could house 4 groups of sows, 4 farrowing units and 6 units for weaners (2 units demanded per batch). The 2 units for fatteners and 2 other units were used to create extra space during the sanitation.

Sows with low appetite or in poor condition were slaughtered. To avoid underestimation of weight, each sow was expected to weight 337 kg and received 1.8 g tylvalosin (Aivlosin®) per os daily for seven days in a previously cleaned unit. Thereafter they were washed and sprayed with Virkon®) and transferred to another cleaned and disinfected unit and treated for another 5 days.

Dry sows were treated batchwise (n=7) from week 46 to week 51 in 2016. Units for dry sows, mating and farrowing were sanitised week 45-50. The first piglets born to sanitised sows were weaned week 1 in 2017. The last non-sanitised growers were sold (30 kg) week 6 in 2017. The weaner units were sanitised week 1-7 in 2017.

The six contaminated fattening herds performed all in-all out production and were informed on the necessity to sanitise their facilities (lifting slatted floors, washing and disinfecting) as they were emptied following slaughter.

All herds were monitored for SD with focus on diarrhoeic pigs.

Results

During the period of 19 months following the second sanitation of the sow herd (November 2016 to June 2018), 72 pigs with loose stool have been tested for presence of *Bhyo* with negative results. Since then (from July 2018), no further samples have been analysed, nor has SD been suspected.

All contaminated fattening herds but one (n=5) have sanitized their facilities following emptying at slaughter. The last herd instead was closed down.

Table 1. MIC:s of four antibiotics for *Bhyo* isolated in the 500-sow herd during 2016. Bolded MIC:s represent resistant isolates

Month	*Tiam*	*Valn*	*Tyl*	*Tval*
Feb	<0.06	<0.03	<2	1
Jun	0.12	0.5	4	1
Aug	**>8**	**>4**	<2	0.5
Oct	**4**	**4**	**>128**	**16**

Tiam = tiamulin; Valn = Valnemulin Tyl = tylosin tval = tylvalosin

Conclusions and Discussion

Initially *Bhyo* was susceptible to tiamulin in the index herd. The resistance to tiamulin (Table 1) was possibly induced by underestimating the weight of sows during the first eradication attempt and/or of growers for sale.

However, *Bhyo* was still susceptible to the on-site none-used substances tylosin/tylvalosin, so a new eradication attempt using high doses of tylvalosin was made and apparently successful. As previously described [2], and as also shown in Table 1, resistance of *Bhyo* to tylvalosin was rapidly induced despite that it was ensured that every sow was given a high dose of the substance. This observation highlighted the need of a perfect plan when sanitizing with tylosin/tylvalosin. The rapid development of resistance to these substances makes the probability to get a second chance poor.

At present, SD has not been diagnosed for three years within the multisite production site, and the eradication appear successful. Thus, Sweden again hopefully is free from tiamulin-resistant SD.

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Swine dysentery – a control program at national level initiated in Sweden

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Introduction

Following the ban of growth promotors effectuated already in 1986, Sweden suddenly experienced problem with Swine dysentery (SD) [1] and studies on SD and on *Brachyspira hyodysenteriae* (*Bhyo*) [2] and on antimicrobial resistance of *Bhyo*[3] were intensified.

Affected herds were sanitized using hygienic measures and tiamulin, and the problems with SD ceased at a national level. Affected herds could suffer severely from the disease [4], but SD was no longer a national problem.

Up to 2016, *Bhyo* was constantly sensitive to tiamulin[5] and sanitation of herds worked well. However, in 2016 Tiamulin resistant SD was diagnosed in a piglet producing herd [6]. This herd was sanitized successfully using tylvalosin, and contaminated fattening herds were thoroughly disinfected when emptied. Thus, Sweden appear to again be free from tiamulin resistant *Bhyo* [6].

However, the incident clearly showed the risk for development of tiamulin resistant *Bhyo*. Therefore, an interest in eradicating SD at a national level arose.

Materials and Methods

SD is a severe but not notifiable disease, so consequently there are no formal regulations regarding SD. Therefore, the first measure undertaken in the aim of eradicating SD on a national level was to establish a network entitled ***SD-free SWEDEN^{NOW}***. The network included the pig farmers organization as owner of the project and the National Veterinary Institute, SVA, as coordinator. The network also included all three national veterinary pig health organizations and all major abattoirs.

In a second step, information of all pig herds diagnosed with SD from 2016 was collected from the members of the network. Herds not yet declared free from SD were merged into a shared register.

The information gained was shared between the stakeholders within ***SD-free SWEDEN^{NOW}*** to be able to identify SD-deemed herds and transport pigs to and from these enterprises as safe as possible, *i.e.* as late in the week as possible.

It was also decided that whenever SD will be suspected, bacteriological samples from at least 10 pigs should be sent to the veterinary institute for analysis of *Bhyo*.

Results

During the period 2016-2020, 25 herds in Sweden were diagnosed with SD (Figure 1). At January 1st in 2020, 16 of these herds had been declared free from SD, and another 3 effectuated sanitation programs and awaited declaration of freedom from SD. The other 6 herds, whereof one was diagnosed 17th of December 2019, had not yet effectuated any sanitation program.

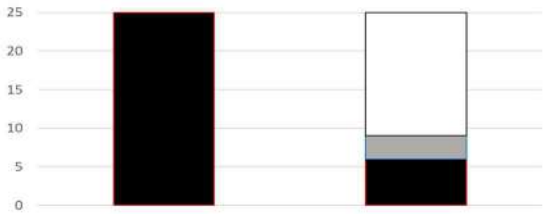

Figure 1. The total number of herds diagnosed with SD during 2016-2019 to the left, and the status of these herds by January 1 2020 to the right (white = declared free from SD; grey = cleansing or sanitized awaiting to be declared free from SD; and black = still with SD).

Conclusions and Discussion

The merged efforts of the network ***SD-free SWEDEN^{NOW}*** have identified all herds diagnosed with SD and certified their status regarding the disease (Figure 1). This information allows to assort diagnosed herds accurately in the transport systems with the aim to protect healthy herds from exposure to *Bhyo*.

The future focus will address the 6 herds still deemed with SD, and any new herd diagnosed with SD, with the aim to sanitize them from the disease. The national status regarding herds with SD will continuously be reported to stakeholders in national media as shown in table 1.

Table 1. SD in Swedish pig herds from January 2020; Status by February 2020

Diagnosed herds				Other herds,	
Diagnosed	**n**	**Status today**	**n**	**tested negative**	**n**
Before 2020	9	Declared free	0	On suspicion	1
From 2020	0	Cleansing	3	--	
		Not sanitized	6	Control	0
TOTAL	**9**		**9**		**1**

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Determination of the optimal timing to vaccinate sows for improved Erysipelas control

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Introduction

Despite the intensive vaccination programs applied in the field, erysipelas continues to be a costly global concern for the swine industry and public health [1,2,3]. Post-farrowing vaccination is routinely applied to protect the breeding stock. However, there is a need to vaccinate growing pigs due to a lack of protection in the finishing period, and it is not uncommon to detect outbreaks in the field due to antibody waning [4,5]. There is a need for further studies that aim at investigating other vaccination protocols that may increase protection against erysipelas under commercial conditions. Therefore, the specific objectives of this study were two-fold: 1) to study the seroconversion in sows and piglets between pre- and post-farrowing protocols against erysipelas over time; and 2) to describe the time-to-negative erysipelas protection between protocols.

Materials and Methods

A total of 35 sows and 68 piglets (2/sow) from a farrow-to-wean farm in the South-East Spain were included in the study. Sows and piglets were assigned into 5 groups considering parity, type of erysipelas vaccine (two commercial vaccines), and vaccination protocol (pre-farrowing vs. post-farrowing) (Table1). Blood samples were collected from sows and piglets at days 7, 14, and 21 of the study, and finally, from piglets at 42, 63, and 84 days. All blood samples were tested for *Erysipelothrix rhusiopathiae* ELISA test and results analysed by a linear mixed model using R software.

Table 1. Study groups, erysipelas commercial vaccine, and protocols studied.

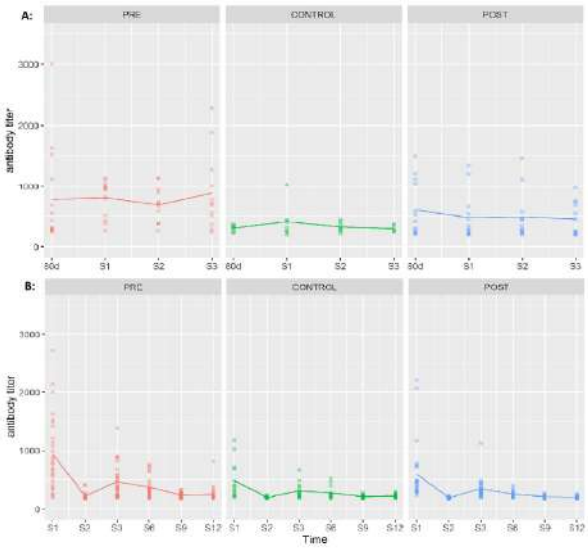
Group	Vaccine	Vaccination protocol
1 (n=7)	Ruvax®	[1]Pre-farrowing (SD -30)
2 (n=7)	Eryseng®	
3 (n=7)	Ruvax®	Post-farrowing (SD +10)
4 (n=7)	Eryseng®	
5 (n=7)	Control	No Vac

[1] SD; Study Day. SD=0 Parity day

Results

For objective 1, the overall mean antibody titer was statistically higher in the pre-farrowing protocol for both sows and piglets; only in piglets, this difference was significant over time (Figure 1) and, most specifically, in piglets from multiparous sows (p=0.0039).

Figure 1. Individual Erysipelothrix rhusiopathiae ELISA antibody titer distribution in sows (A) and in piglets (B), per week of sampling by vaccination protocol



For objective 2, time-to-negative status was almost significant between sows of different protocols (p=0.049). Piglets from the post- protocol became negative earlier than those in the pre-farrowing protocol (p=0.0037).

Conclusions and Discussion

Results from this study demonstrated that a pre-farrowing vaccination protocol provided longer protection to piglets. Our results showed that a pre-farrowing protocol provides higher erysipelas' serum antibodies levels during lactation overtime that translated in longer protection to piglets against future challenges.

Acknowledgments

The authors would like to thank the farmer and farm staff for their help during the study.

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Comparative Virulence and Genomic Analysis of *Streptococcus suis* Isolates

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Introduction

Streptococcus suis is a bacterial swine pathogen causing substantial economic and health burdens to the pork industry. Mechanisms used by *S. suis* to colonize and cause disease remain unknown and vaccines and/or intervention strategies currently do not exist. Studies addressing virulence mechanisms used by *S. suis* have been complicated because different isolates can cause a spectrum of disease outcomes ranging from lethal systemic disease to asymptomatic carriage. The objectives of this study were to perform comparative genomic analyses of *S. suis* isolates that exhibit different pathogenic capacities to identify genomic attributes associated with virulent phenotypes.

Material and Methods

Nine isolates obtained from within the U.S. were chosen for evaluation in this study. Virulence of the isolates, along with a virulent reference strain P1/7, was assessed by evaluating morbidity and mortality following intranasal challenge of Caesarean-derived, colostrum-deprived (CDCD) pigs. Whole genome sequencing was then performed using Pacific Biosciences (PacBio) and Illumina MiSeq platforms. Closed whole-genome assemblies were generated using the PacBio smrtanalysis, CANU, or Unicycler. The Illumina MiSeq paired-end sequencing reads were used for polishing and error correction of the assembled closed genomes with Pilon. Further comparative analysis was performed using Mauve, HMMER, BLASTN, BLASTX, ISfinder, IslandViewer, and PHASTER. ResFinder 2.1 and the Comprehensive Antibiotic Resistance Database (CARD) were employed for identification of antimicrobial resistance elements.

Results

Two *S. suis* isolates (ISU2614 and ISU1606) exhibited a high level of virulence, similar to P1/7, with all pigs (5 out of 5) in each of these groups developing systemic clinical disease within 8 days post-challenge. Three *S. suis* isolates (ISU2714, ISU2660, and ISU2514) were moderately virulent with 3 out of 5 pigs challenged with ISU2714 developing neurologic signs and/or lameness, while only 2 out of 5 pigs challenged with ISU2660 developed lameness. 1 out of 5 pigs challenged with ISU2514 developed neurologic signs and 2 out of 5 developed lameness. Four *S. suis* isolates (ISU2414, ISU2812, ISU2912, and SRD478) were completely avirulent and all pigs in these groups remained healthy and exhibited no signs of clinical disease. Nucleotide diversity of 45 genes encoding proposed virulence factors were evaluated by comparing the average percent identity relative to the reference strain P1/7. Overall, the highest sequence divergence was observed in the genes from the non-virulent isolates (ISU2414, ISU2812, ISU2912, and SRD478). Gene content among all isolates was analyzed and allowed for the determination of core genes (present in all 10 isolates), accessory genes (present in 2-9 isolates), and unique genes (present in in only one isolate). Four types of capsule loci were observed among the isolates in this study with the majority of harboring a Type 2 capsule (P1/7, ISU2614, ISU1606, ISU2714, ISU2660, ISU2514, and ISU2414). Serotypes for the other isolates were defined as serotype 19 for ISU2812, serotype NCL1-3 for ISU2912, and the serotype of SRD478 was found to be undefined and highly similar to the capsule loci from other non-typeable *S. suis* isolates.

Conclusions

Whole genome sequencing followed by comparative genomic analyses revealed several notable regions of difference, including regions encoding secreted and membrane-associated factors, which likely contributed to the spectrum of clinical disease observed. Collectively, these results provide a foundation for understanding the genomic attributes responsible for the spectrum of virulent phenotypes that exist among *S. suis* isolates.

Localization of pathogenic *Streptococcus suis* strains in tissues by *in situ* hybridization

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Introduction

Streptococcus suis is a Gram positive bacterium common in the respiratory tract of pigs as a commensal organism but pathogenic strains are known to cause systemic and neurologic disease (2). To diagnose *S. suis* infection, it is important to distinguish commensal from pathogenic strains isolated from porcine tissues. One method of subtyping *S. suis* is multi-locus sequence typing of 7 housekeeping genes yielding a unique sequence type (ST). *S. suis* ST1 has been predominately isolated from clinical cases, and according to recent data, ST28 isolates in the U.S. are likely pathogenic (1). To further characterize and diagnose a pathogenic strain of *S. suis*, the strain must be observed in association with lesions of porcine tissues. This can be accomplished by *in situ* hybridization (ISH) with the use of an RNA probe. The objective of this study was to perform ISH with RNA probes specific to virulence associated genes (VAGs) of known pathogenic strain ST1 and novel pathogenic strain ST28 to demonstrate positive signal in respective affected tissues.

Materials and Methods

Using the distribution of published *S. suis* VAGs from a previously characterized sample set (1), candidate genes were selected based on their prevalence and specificity to ST1 and ST28 strains. Sequence alignments were generated to select the best targets to design an RNA probe for use with RNAscope ISH (Advanced Cell Diagnostics, Newark, CA). An RNA probe targeting 16S rRNA of eubacteria was also used as a positive control (3) and a no-probe negative control measured the level of nonspecific staining. Briefly, the ISH protocol included pretreatment, probe hybridization, amplification, addition of red chromogen, and counterstain.

Results

Positive signal for *S. suis* ST1 in brain-1 tissue was observed in a meningeal lesion (Figure 1) in association with inflammatory infiltrates.

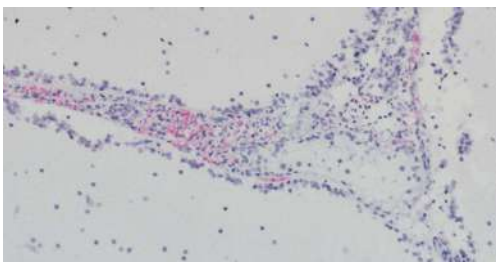

Figure 1: Positive staining (red) for ST1 probe in meninges of brain-1

Staining for the remaining tissues and their respective ST probes was not observed for either *S. suis* ST1 or ST28 (Table 1).

Table 1. Tissues, ST of *S. suis* isolated, probe, and ISH result.

Tissue	ST of isolate	Probe	Signal
Brain-1	1	ST1	Pos
		Eubact.	Pos
Brain-2	1	ST1	Neg
		Eubact.	Pos
Heart-3	28	ST28	Neg
		Eubact.	Pos
Heart-4	28	ST28	Neg
		Eubact.	Pos

All 4 tissues showed positive staining with the eubacteria probe while nonspecific staining was not observed in any of the tissues with the no-probe control.

Conclusions and Discussion

The presence of positive signal in tissue can validate whether or not a particular strain of *S. suis* is associated with histological lesions and causing clinical disease. This enables confirmation of a diagnosis and facilitates choice of strains for autogenous vaccine production. In the case of brain-1 tissue, ISH signal was observed within the meninges as expected, which gives further assurance that the probe is specific for *S. suis* ST1 isolated from that tissue. The lack of signal in the other tissues, especially the brain-2 tissue from which an ST1 *S. suis* strain was also isolated, may be attributed to ineffective pretreatment of tissues. Further, since the probes are RNA probes, there must be sufficient expression of the target sequence and perhaps expression of the targeted VAGs in certain tissues or by certain strains is low.

Acknowledgments

Danielle Sardeson and the University of Minnesota Histology Laboratory

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Distribution of *Glaesserella* (*Haemophilus) parasuis* serotypes on farms with recurring respiratory diseases prior and after antibiotic treatments

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Introduction

Glaesserella parasuis (*GPS*), formerly *Haemophilus parasuis*, is a colonizer of the upper respiratory tract of pigs, but under specific conditions, it can lead to severe systemic diseases or pneumonia (1,2). Not only weaners but also adult pigs like gilts or sows can be affected and may show clinical signs (3). The interpretation of the aetiological involvement of *GPS* in pneumonic diseases is complicated by the fact that different results exist regarding the pathogenicity of serotypes and that limited information about virulence factors is available. Therefore, in the current study the occurrence of *GPS* serotypes and their proportion harbouring virulence-associated trimeric autotransporters (vtaA) was investigated on 20 piglet producer farms with problems due to recurring respiratory diseases in nursery pigs.

Materials and Methods

The piglet producer farms were chosen based on their level of recurring respiratory disease outbreaks during the last years and therefore increased levels of antibiotic treatment. The 20 farms were visited twice, before (visit A) and after (visit B) an antibiotic treatment due to a respiratory disease outbreak. During each visit, ten affected pigs were clinically examined and from three pigs bronchoalveolar lavage fluid samples were taken. These samples were examined for the occurrence of G*PS* by bacteriological culture and PCR (4) and antibiotic sensitivity of the isolates from the bacteriological culture was tested . Serotyping and determination of vtaA leader sequence (LS) as virulence factors of *GPS* isolates was performed by PCR (5,6).

Results

In total 24 outbreaks of respiratory diseases were analysed. The respiratory health status at visit A revealed 1.3% of the pigs with no, 96.2% with mild, and 2.5% with moderate respiratory symptoms. At visit B 22.7% of the pigs showed no, 76.9% mild and 0.4% moderate symptoms. At visit A G*PS* was cultured from 79.7% of the BALF samples, at visit B from 81.3%. PCR results were similar. Of the G*PS* isolates none (visit A) and 9.6% (visit B) were resistant to the used antibiotics. Serotyping yielded identical results before and after antibiotic treatment in 43.8%. The most prevalent serotypes in this study were serovars 4 (21%), 7 (21%), 5/12 (14.3%) and 13 (11.8%), serovars 9, 11 and 14 were not identified. 6.7% of G*PS* isolates were not typeable. Applying the vtaA-LS PCR, respectively 83.3% (visit A) and 85.1% (visit B) of the isolates were positive. The concordance of results from bacteriological culture and PCR showed a correlation of 70.0% for visit A and 74.1% for visit B. There was no significant correlation between the detection results of *GPS* and vtaA-positive *GPS* isolates and the changes in respiratory health status at both examination days. Nevertheless, there was a significant correlation between the reduction of *GPS* isolates and vtaA-positive *GPS* isolates (p: <0.0001).

Conclusions and Discussion

In this study the chosen examination method (culture, PCR) has a significant impact on the detection rate of G*PS*. Additionally, the occurrence of *GPS,* as well as the detection rate of potentially virulent isolates was not influenced by the respectively applied antibiotic treatment based upon general pneumonic symptoms. Regarding these results, the question arises, if the antibiotic treatment can be considered successful or even necessary. The comparable detection rate of G*PS* isolates at both time points of sampling should be critically mentioned when the use of antibiotics on farms with recurring respiratory diseases and evidence of *GPS* is considered. A correlation between the occurrence of an underlying infection with potentially virulent G*PS* strains and persistent problems in herds with respiratory diseases can be assumed.

Acknowledgments

The VASIB consortium would like to thank the Federal Ministry of Food and Agriculture (BMEL) via the Federal Office for Agriculture and Food (BLE) for funding.

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Characterization of *Actinobacillus pleuropneumoniae* isolates from German pigs

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Introduction

In 2017 and 2018 Bossé et al. described new serotypes of *Actinobacillus (A.) pleuropneumoniae* and established a new multiplex PCR detecting all known serotypes [1, 2]. In the present study, this method was applied to a collection of recent *A. pleuropneumoniae* isolates from Germany in order to get an insight into the current distribution of serotypes. Additionally, isolates were also characterized with respect to their Apx toxin gene profile to assess their virulence.

Materials and Methods

Bacterial strains were collected from submissions to the clinical microbiology lab of the Institute for Microbiology, University of Veterinary Medicine, Hannover. A total of 222 *A. pleuropneumoniae* strains isolated between 2010 and 2019 were characterized.
A. pleuropneumoniae was serotyped using the multiplex-PCR described by Bossé et al. that includes all currently known serotypes [2]. Additionally, isolates were typed with respect to their Apx toxin type [3].

Results

The majority of *A. pleuropneumoniae* isolates was represented by serotype 2 (64%) and serotype 9/11 (15.3%). The remaining 16.7% of isolates belonged to 8 different serotypes, whereas 4.1% of the isolate collection were non-typable (Fig. 1). Interestingly, two isolates belonged to serotype 16 and seven isolates appertained to serotype 18, which both were only described recently [1, 4].
The PCR also included primers for the *NadV* gene, but only one isolate proved to be biovar 2. Interestingly, this isolate was positive for *apxIIICA* and *apxIIIBD*, and belonged to serotype 18. In an isolate collection encompassing 5 isolates from before 2010, two additional isolates belonged to biovar 2 and were typed as serotype 2. These two isolates were lacking the *apxIIICABD* operon.
For biovar 1 isolates, Apx toxin gene profiles usually corresponded to expected profiles for certain serotypes. However, 5.9% of the isolates showed unusual profiles including 4 of 6 isolates of serotype 18 (Fig. 2). These serotype 18 strains (as their biovar 2 counterpart) only possessed *apxIIICA* and *apxIIIBD*.

Conclusions and Discussion

The last published reports on serotype distribution of *A. pleuropneumoniae* in clinical samples from Germany date back to the 1990ies and revealed a predominance of serotype 2 and to a lesser extent serotype 9. This obviously has not changed since then despite the availability and application of a serotype 2 specific vaccine. Whether serotype 16 and 18 have newly emerged or were concealed in the fraction of previously non-typable isolates is unclear. Biovar 2 isolates seem to be very rare and, in discordance to the general assumption that biovar 2 isolates are lacking ApxIII, the serotype 18 biovar 2 isolate did possess an *apxIIICABD* operon.

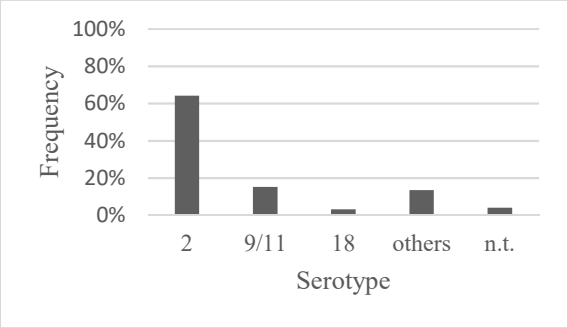

Figure 1. Distribution of serotypes (n=222).

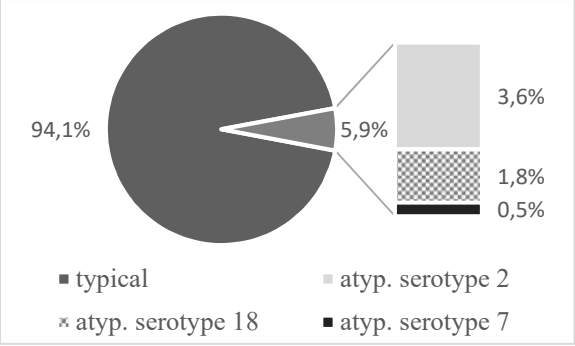

Figure 2. Apx toxin gene profiles of biovar 1 (n=221)

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Characterization of *Glaesserella parasuis* isolates from German pigs

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Introduction

Over the last years new serotyping methods and new pathotyping protocols have been published for *Glaesseraella (G.) parasuis*. This study applies some of these new lab tools to characterize a large collection of German isolates of *G. parasuis* from clinical cases as well as isolates from non-clinical carrier animals.

Materials and Methods

Bacterial strains were collected from submissions to the clinical microbiology lab of the Institute for Microbiology, University of Veterinary Medicine, Hannover between 2012 and 2019. A total of 286 *G. parasuis* isolates were characterized. Isolates were grouped based on anamnestic information into isolates from systemic disease (Glaesser´s disease, n=53, pulmonary infections (n=204) and nasal swabs (carrier, n=29).
G. parasuis was serotyped implementing the multiplex-PCR published by Howell et al. [1] as well as primers published by Jia et al. [2] in different monoplex PCRs. Moreover, selected isolates were also serotyped with a PCR using primers modified from the Howell paper [3] to account for inconsistencies to conventional serology.
For pathotyping of *G. parasuis* isolates the LS-PCR designed by Galofré-Milà et al. [4] was compared with a multiplex-PCR described by Howell et al. ("pathotyping-PCR") [5]. Data was analyzed using Fisher´s exact test with $p<0.05$.

Results

In *G. parasuis* distribution of serotypes was quite diverse (Fig 1). Serotypes 6 ($p<0,001$) and 9 ($p=0.007$) were overrepresented in carrier isolates. Seven % of the isolates showed inconsistent results between the different methods applied for serotyping, pertaining mainly serotype 1, 2, 11 as previously described [3].
Using the LS-PCR systemic isolates were significantly more often classified as virulent ($p<0.001$), whereas typing as non-virulent was significantly associated with carrier isolates ($p<0.001$).
Pathotyping profiles obtained from the multiplex-PCR by Howell et al. are computed in R to result in a score-value classifying isolates as virulent, potentially virulent (boundary) and non-virulent. Results were comparable to results from LS-PCR (Fig 2).

Conclusions and Discussion

Molecular serotyping of *G. parasuis* still is challenging and results using different protocols are not always in accordance. Only few serotypes seemed to be associated with carrier status while the majority of serotypes was isolated from carrier and diseased animals. Therefore, methods for pathotyping are needed to identify (more) virulent isolates. The LS-PCR is easy to perform and evaluate and correlated well with the origin of isolates ("silver" standard) in our sample of isolates. Pathotyping as described by Howell et al. is complex and needs computing of a score for classification.

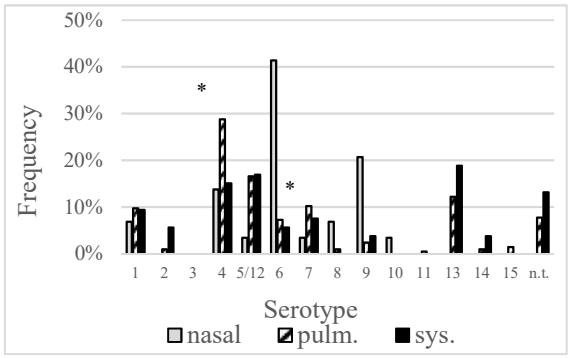

Figure 1. Distribution of serotypes (n=286).

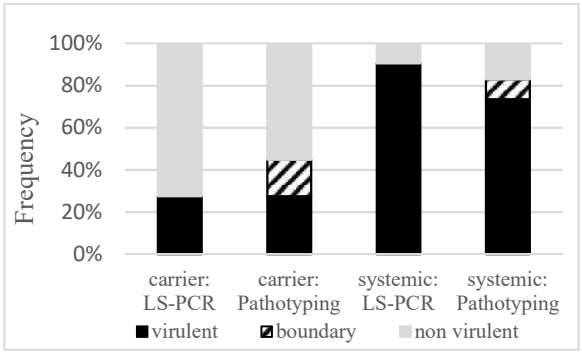

Figure 2. Comparison of LS-PCR and pathotyping PCR (n=286).

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Identification of four candidate virulence-associated genes of *Streptococcus suis* isolates from the United States

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Introduction

Streptococcus suis is a significant economic and welfare concern in the swine industry. Pathogenic strains are associated with systemic and neurological disease in growing pigs (1) while commensal strains normally reside in the upper respiratory tract.

In a previous study, we characterized 208 *S. suis* isolates collected across the United States by subtype and pathotype (pathogenic, possibly opportunistic, and commensal) (2). Investigation of associations between subtype and pathotype classifications identified multiple subtypes that corresponded to the pathogenic pathotype, such as serotype 1/2 and multilocus sequence type (ST) 28, which were the most predominant U.S. subtypes identified.

In the current study, we applied pan-genome analysis to the 161 U.S. *S. suis* isolates belonging to the pathogenic (n=139) and commensal (n=22) pathotypes (2). Our objective was to identify accessory genes that associated with the pathogenic pathotype and thus may serve as novel candidate virulence-associated genes (VAGs) of *S. suis*. Further, the identification of such candidates may elucidate a VAG panel for the prediction of pathogenicity of *S. suis* isolates in the United States.

Materials and Methods

The 161 *S. suis* genomes were assembled into draft genomes, annotated, and the pan-genome (core and accessory genes) was determined. Accessory genes illustrating a significant association with pathotype (p-value<0.05, <50% commensal, >50% pathogenic) were further analyzed by a Least Absolute Shrinkage and Selection Operator (LASSO) regression model to identify the fewest number of genes that could be included in a panel to differentiate between pathogenic and commensal isolates. The ability to identify pathogenic isolates using this panel was verified using a second sample set of *S. suis* isolates, which consisted of 21 pathogenic and 13 non-pathogenic isolates from a single system.

Results

A total of 1,245 core and 5,361 accessory genes were identified in the pan-genome. The 229 accessory genes that illustrated a significant association with pathotype were further analyzed by the LASSO model to investigate these candidate VAGs for use in a panel predictive of pathotype. The pan-genes 2859, 3482, 3448, 4779 were identified as having a significant association with pathotype. The profile was observed in 84% (135/161) of *S. suis* isolates, from which 98% (132/135) were classified as the pathogenic pathotype. These results can be interpreted as a 98% probability that an isolate is pathogenic given the profile.

Table 1. Pathotypes of *S. suis* isolates that are positive for the novel VAG panel consisting of pan-genes 2859, 3482, 3448, 4779.

	No. of isolates containing the pan-gene panel (n=135)	No. of isolates not containing the pan-gene panel (n=26)
Pathogenic (n=139)	132 (97.8%)	7 (26.9%)
Commensal (n=22)	3 (2.2%)	19 (73.1%)

The *S. suis* isolates with this candidate VAG profile belong to pathogenic subtypes (2), including serotypes 1, 1/2, 2, and 7, and STs 1, 28, 94, and 108. Using this same approach, we were able to identify 80% of pathogenic isolates in the second sample set.

Conclusions and Discussion

This study demonstrated the use of a pan-genome approach to identify novel candidate VAGs of *S. suis* isolates in the United States which may serve as novel gene targets for virulence studies. We further investigated the utility of a novel panel of three candidate VAGs as a pathotyping method for *S. suis*. Using this novel VAG panel, we were able to identify the prevalent *S. suis* subtypes in the United States, including serotype 1/2 and ST28, which are associated with systemic and neurological disease (2). We further verified the utility of this panel for differentiating pathogenic from non-pathogenic isolates within a single system. These findings may inform novel diagnostic approaches for enhancing *S. suis* control in the United States.

Acknowledgments

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Using qPCR and serology to asses a high performing finishing pig herd's subclinical ileitis status

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Introduction

Ileitis is present in most commercial finishing pig barns in Holland (1). Serology only proves infection and necropsy is considered as the golden standard for diagnosing Ileitis due to infection with *Lawsonia intracellularis (Li)*. However, when dealing with subclinical disease, it is hard to select animals for necropsy. Over the recent years, *Li* qPCR testing makes it possible to detect infections with *Li* and to quantify the severity by the amount of *Li* present in the feces (2). The objective of this study is to check and quantify the presence of ileitis due to infections with Li by combining serology and qPCR without clinical signs of ileitis.

Materials and Methods

A 4,500 head finishing pig barn (TN70 x Tempo; gilts and boars) with above average management and biosecurity practices was selected. ADG of the farm is above the Dutch average (945 vs 840 gram/day). Pigs were allocated in the finishing pig farm at an age of 10 weeks (25.2 kg) with 50 pigs/pen and a floor surface of 0.9 m^2/pig. Pigs were slaughtered at 121 kg live weight after 101 days finishing.

Eighteen rooms were followed on a 4 weekly basis. Environmental samples were taken at 4, 8 and 12 weeks of finishing from the same 2 pens by using a surroundings swab (Solar biological) and were tested with a quantitative PCR for the presence of *Li* bacteria. Additional 4 pigs per room were bled at 4 and 8 weeks finishing and were tested for antibodies against Li. Tests were performed in the Bioscreen Laboratory, Hannover, Germany.

Results

The qPCR results showed a clear presence of *Li* with 74% positive samples at 4 weeks after arrival. Presence of Li in surrounding swabs went down in later finishing to 38% at 8 weeks and 3% at 12 weeks after arrival (Table 1)

Table 1 qPCR Li sampling results of the surrounding swabs.

Li qPCR testing (log GE/ml)	Weeks after arrival		
	4	8	12
# Samples	34	40	40
# Positive	25	15	1
% Positive	74%	38%	3%
Mean qPCR	5.27	5.61	3
Min	3	3	3
Max	7.08	7.29	3
St.dev.	1.28	1.12	n/a

Elisa antibody testing showed a similar profile, with 49% positive samples at 4 weeks after arrival.

Table 2 Li Antibody results (Svanovir AB Elisa)

Antibody Elisa results	Age at sampling (weeks finishing)	
	4	8
# Samples	68	76
Negative	31 (45%)	11 (14%)
Ambiguous	4 (6%)	3 (4%)
Positive	33 (49%)	62 (82%)

Conclusions and Discussion

Despite the fact that this farm has a very high ADG of 945 and a very efficient FCR of 2.25, numerous positive environmental swabs could be detected, mainly in the starter phase. From in total 42 positive qPCR samples, values were mainly <log 6 GE/ml in 24/42 samples (57%). Values between log 6 and log 7 GE/ml are associated with a small reduction in ADG of 15 gram/day (2), and were found in 15/42 samples (36%). Samples >log 7 GE/ml are associated with a larger growth depression in ADG (2) but were only found in 3/42 samples (7%) in this case.

Based on serological results, the most likely time of start of infection is direct after allocation considering 3 weeks of time before pigs turn positive with the use of an Elisa (3).

Based on the combination of serology and moderate positive qPCR results, it is to be concluded that the herd suffers from subclinical ileitis during the early starter and grower phase.

If oral vaccination against Lawsonia is considered at this farm, it should be at a minimum of 3 weeks before the estimated infection time point considering an onset of immunity of 3 weeks after vaccination.

Evaluation of vaccination is advised based upon zoo-technical parameters like ADG, FCR and mortality .

Acknowledgments

Thanks to Boehringer Ingelheim Animal Health the Netherlands for financing the diagnostics.

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Evaluation of finishing pig farm at slaughter for Ileitis due to infections with Lawsonia intracellularis: preliminary results

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Introduction

Infections with *Lawsonia intracellularis* (*Li*) are quite common in pigs. Ilea at slaughter can be investigated without the need to sacrifice animals. However, the presence of ileitis at slaughter is poorly investigated and mostly is only performed with macroscopic evaluation. The use of a *Li* qPCR on ileum tissue is a good alternative for immunohistochemistry (IHC) (1). The objective of this study is to evaluate the presence of ileitis at slaughter, confirmed by qPCR testing and includes the preliminary results of this ongoing project

Materials and Methods

Veterinarians in the Netherlands were asked for farms with moderate to below average performance when looking at FCR and/or ADG. At slaughter 40 ilea were collected. From the first 10 ilea, samples were taken (at random selected ilea; ARI). The remaining 30 ilea were first evaluated macroscopically. Ten ilea with the most apparent visible lesions were subsequently selected and sampled (Macroscopic selected ilea, MSI). Approximately 10 cm from the ileocecal junction a 0.5 cm sample of ileum tissue was taken for qPCR analysis. Samples were analyzed by *Li* qPCR (Bioscreen Hannover). Based on the results of the first 15 farms it was decided to continue using only 10 ARI.

Results

In total 376 ilea obtained from 23 different farms were analyzed using qPCR (Figure 1). Out of these 23 farms, seven farms tested negative for all PCR samples. From five farms, all selected ilea were positive by *Li* qPCR. When qPCR results from ARI and MSI from the same farm were compared (excluding negative results), ARI were significant lower in the amount of present Lawsonia intracellularis bacteria (ARI: 5.40 log GE/ml; n=48 vs MSI: 6.61 log GE/ml, n = 48; p< 0.0001). However, detection of presence of *Li* in the ilea was not different between ARI and MSI selected ilea per farm. Two farms had clinical symptoms of ileitis present in the barn, all of these ileum samples were high positive at slaughter, (mean 6.29 log GE/ml and 6.24 Log GE/ml).

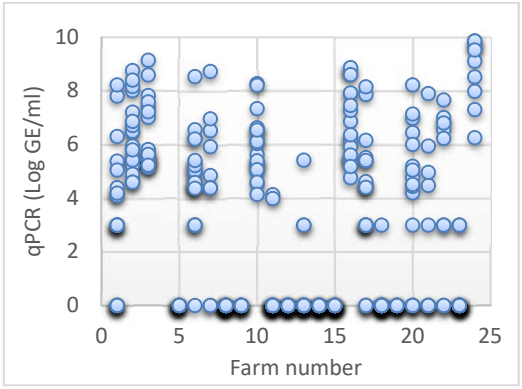

Figure 1 qPCR results per farm. Values >log 6 GE/ml are correlated with positive IHC (1)

Additional to the samples taken for this study, also 10 ilea were investigated which were not suitable for the production of intestinal casings for sausage production and were discarded by employees of the slaughterhouse. The loss of quality of these intestines was due to either eosinophilic enteritis (probably due to parasitic infections) or due to infections with *Li* resulting in porcine intestinal adenomatosis.

Conclusions and Discussion

The evaluation of intestines at slaughter could be a useful tool in assessing the intestinal health status of finishing pig farms without the need to sacrifice animals for necropsy. Only macroscopic evaluation of ilea is not enough to diagnose ileitis and further confirmation by laboratory testing is needed. This is the first report of the use of *Li* qPCR taken from ileum samples at slaughter. The random selection of only 10 ilea is sufficient to asses a herds ileitis status. However, more research is needed to confirm if early *Li* infections are detected at slaughter as well and if the assessment of only one batch of pigs is sufficient to predict the ileitis status at a farm over a longer period in time.

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Antibiotic susceptibility of *Streptococcus suis* in Swedish grower pigs

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Introduction

Streptococcus suis is one of the most important bacterial pathogens affecting pig production worldwide. In Sweden, *S. suis* has not received a lot of attention until relatively recently, and the objective of this study was to investigate the antibiotic susceptibility of isolates from herds with a history of *S. suis* infections as well as from herds without diagnosed cases.

Materials and Methods

Swab samples were collected during 2018-2019 from the palatine tonsils of 200 apparently healthy pigs 8-13 weeks of age. The pigs were from 20 commercial farms around Sweden, ten of which had experienced clinical signs in pigs consistent with *S. suis*, and ten of which had not. Swabs were cultured on colistin-oxolinic blood agar (COBA) at 37°C in 5% CO_2 overnight. Colonies resembling *S. suis* (based on morphology and the presence of α- or β-hemolysis) were pure-cultured on horse blood agar at 37°C in 5% CO_2 overnight and identified to the species level using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) with a cutoff score of 2.0. The antimicrobial susceptibility of 188 isolates was assessed by broth microdilution, using commercially prepared VetMIC GP-mo panels (National Veterinary Institute, Uppsala, Sweden). MIC values were interpreted using cutoffs according to Swedres-Svarm (1).

Results

The prevalence of *S. suis* in the sampled pigs was 95%. Antibiotic susceptibility testing revealed that 13.0% of isolates were non-susceptible to penicillin, and that 99.5 % of isolates were non-susceptible to tetracycline (Table 1).

Conclusions and Discussion

This study shows that *S. suis* is more common in Swedish pigs than previously estimated, and for the first time reports the presence of Swedish isolates non-susceptible to penicillin. Sweden has a very low level of antimicrobial usage in food-producing animals, and benzylpenicillin is the most common substance sold for use in pig production (2). In 2014, 75% of antibiotics sold for pigs were for injection, and 60% of those were products containing benzylpenicillin (2).

Compared to previous studies (1, 3) a higher percentage of isolates was found non-susceptible to tetracycline. However, care should always be taken when making comparisons between studies employing differing methodologies and interpretive criteria.

Acknowledgments

Robert Söderlund (SVA), Lise-Lotte Fernström (SLU).

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Table 1. MIC distribution (%) of *Streptococcus suis* isolates from the tonsils of 200 healthy grower pigs in Sweden.

Antimicrobial agent	Non-susceptible (%) 2018-2019	*Streptococcus suis* (n=188) Distribution (%) of MICs (mg/L)											
		≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥64
Cefalotin	11.2%			2.7	17.6	19.7	29.8	19.1	10.1	1.1			
Cefoxitin					4.8	2.7	8	11.7	18.6	54.3			
Chloramphenicol					0	3.7	1.1	31.9	61.2	2.1	0	0	
Ciprofloxacin				0.5	16	59.6	17.6	4.8	1.6				
Clindamycin	68.6%				26.6	4.8	2.1	2.7	4.8	20.2	7.4	31.4	
Enrofloxacin				0.5	13.3	59.6	22.9	3.7	0				
Erythromycin	35.2%				62.7	2.1	1.1	2.1	4.8	1.6	2.7	22.9	
Gentamycin				0.5	0.5	6.9	32.4	36.7	21.8	1.1	0		
Penicillin[a]	13.0%	26.6	27.7	17.9	14.7	9.2	2.7	1.1	0				
Tetracycline	99.5%					0.5	11.2	22.9	21.3	6.9	1.1	29.3	6.9
Trimethoprim					60.6	2.7	4.8	5.9	5.9	5.9	14.4		
Trim/Sulf[b]	18.6%			69.7	5.9	5.9	5.9	9	1.6	2.1			

[a] n=184 in the case of penicillin. [b] Concentration for trimethoprim given, tested in combination with sulfamethoxazole in a concentration ratio of 1:19.

Prevalence of virulence factors of *Escherichia coli* isolated from piglets with post-weaning diarrhoea in United Kingdom and the Republic of Ireland.

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Introduction

Post-weaning *Escherichia coli* (*E. coli*) diarrhea (PWD), also called post-weaning enteric colibacillosis, remains a major cause of economic losses for the pig industry (1,2). PWD typically causes mild to severe watery diarrhea between 5 and 10 days after weaning and is caused primarily by enterotoxigenic *Escherichia coli* (ETEC). The most common adhesins found on ETEC from PWD in pigs are associated with fimbriae F4 (previously called K88) and F18, while the predominant enterotoxins are heat-labile toxin (LT), heat-stable toxin a (STa), and heat-stable toxin b (STb). In addition to F4 and F18, other fimbrial adhesins, such as F5 (K99), F6 (987P), and F7 (F41), have been associated with PWD, but less frequently (3-6). The objective of the present study was to determine the prevalence ETEC subtypes causing PWD in United Kingdom and Republic of Ireland (UK&ROI).

Materials and Methods

Fifty pig herds distributed throughout UK&ROI showing clinical signs of PWD were sampled (Jan 2018 - Dec 2019). Rectal swab samples (n = 5) from diarrheic pigs were submitted to IZSLER (Brescia, Italy). Following bacterial culture on a blood agar, colonies were picked for further PCR analysis on the presence of different virulence factors - adhesins (F4, F5, F6, F18 and F41) and toxins (LT, STa, STb, Stx2e).

Results

In total, 148 samples were cultured on blood agar. Seven plates (4.7%) had no bacterial growth, whereas 48 non-hemolytic (32.5%) and 93 hemolytic (62.8%) *E. coli* strains were isolated. Both hemolytic and non-hemolytic *E. coli* strains were submitted for PCR testing. Seventy-six strains (51.0%) had no virulence factors. The prevalence of the different ETEC subtypes within the strains positive for virulence factors was as follows: F18-ETEC (24.8%), F4-ETEC (12.8%), F4-F18-ETEC (2.0%) and F18-STEC (2.0%). On a herd level, the prevalence of the different ETEC subtypes was as follows: F18-ETEC (42.4%), F4-ETEC (21.2%), F4-F18-ETEC (3.0%) and F18-STEC (3.0%). Ten farms (30.3%) had a mixed infection with different pathotypes. Table 1 shows the distribution of different virotypes among the 74 virulent *E. coli* strains identified.

Table 1. Distribution of virotypes among the 74 virulent *E. coli* strains isolated on UK&ROI pig farms.

Virotype	Number of strains	% of total
F18STaSTb	26	35.1%
F18STaSTbStx2e	7	9.5%
F18STbLT	6	8.1%
F4LT	4	5.4%
F4STaSTbLT	4	5.4%
F4STbLT	4	5.4%
F18Stx2e	3	4.1%
F4STa	3	4.1%
F4STaSTb	3	4.1%
F18STa	2	2.7%
F18STaSTbLT	2	2.7%
F18STbStx2e	2	2.7%
F4-F18	2	2.7%
F4F18STb	2	2.7%
F18STb	1	1.4%
F41STb	1	1.4%
F4F18STaSTb	1	1.4%
F4STb	1	1.4%

Conclusions and Discussion

This study confirms that fimbriae type F18 was twice as prevalent as F4 among *E. coli* isolates from PWD cases in UK&ROI. Laboratory diagnostics, including characterization of virulence factors, are essential to understand the role of *E. coli* in PWD outbreaks and initiate appropriate preventive measures such as a live oral *E. coli* vaccination.

References

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5. Chen et al., 2004. Vet. Microbiol. 103, 13-20.
6. Vu Khac et al., 2006. BMC Vet. Res. 2, 10

Keywords

ETEC – virulence factors – *Escherichia coli* – post-weaning diarrhea.

Brachyspira hyodysenteriae eradication with a novel non-antibiotic zinc chelate: the approach of the future?

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Introduction

Swine dysentery caused by *Brachyspira hyodysenteriae* (*B. hyodysenteriae*) is an important intestinal disease with clinical signs typically consisting of muco-haemorrhagic diarrhea. Economic losses are due to mortality, diminished growth rates, deterioration of feed conversion and cost of medical treatment. Diagnosis is performed using pooled faecal samples (microbial culture, PCR test). A novel non-antibiotic zinc chelate has been reported to demonstrate positive effects on fecal quality and consistency, general clinical signs, average daily weight gain and *B. hyodysenteriae* excretion after a 6-day oral treatment (1,2). The objective of the study was eradicate *B. hyodysenteriae* from closed swine herd using a novel zinc chelate (Intra Dysovinol®; Intracare BV) in combination with a cleaning and disinfection (C&D) protocol, including rodent and fly control, manure management and improved external and internal biosecurity rules.

Materials and Methods

A closed swine herd with 240 sows in a one-site production system had been diagnosed positive for *B. hyodysenteriae* in December 2018. The isolated strain showed strong resistance to all currently used antibiotics for eradication purposes. Nevertheless, a first eradication was carried out in Spring 2019 that failed due to Ab-resistance and inconsistent internal biosecurity measures. Subsequently, an audit - to identify critical control points to be improved - was performed. Several steps in C&D, rodent and fly control and biosecurity issues were optimized before a new eradication was started. A second eradication was initiated using a novel zinc chelate for oral drinking water application (1,2), which has been proven efficacious in eliminating *B. hyodysenteriae* from the pigs following a 6-day continuous drinking water administration.

Results

The entire sampling and treatment schedule with subsequent results is given in Table 1. Overall, 3 sampling points were identified to determine the *B. hyodysenteriae* infection status of the animals in scope for the zinc chelate treatment. Following treatment, in combination with C&D, all animals tested PCR-negative and remained negative until the end of the protocol. Further follow-up over the next months will be needed to confirm long-term success of the eradication efforts.

Conclusions and Discussion

The current case report describes *B. hyodysenteriae* eradication with a novel non-antibiotic approach using a novel zinc chelate dosed through the drinking water. Earlier studies have demonstrated efficacy of the zinc chelate to clear *B. hyodysenteriae* from the pig after a 6-day treatment (1,2).

Table 1. Sampling and treatment protocol for *B. hyodysenteriae* eradication using a novel zinc chelate product. D0 – day of initiation of treatment

Time-point	*Activity description*	*Results*
d-3	Fecal sampling (n = 21) prior to treatment	3 / 21 (14.2%)
d0	Start of treatment with novel zinc chelate through drinking water	
d6	Fecal sampling (n = 21) after 6 days treatment	0 / 21 (0%)
d7	Start of C&D protocol, combined with sow washing and movement	
d10	End of C&D protocol	
d15	End of treatment with novel zinc chelate through drinking water	
d16	Fecal sampling (n = 21) after end of treatment	0 / 21 (0%)

In the current eradication protocol, treatment was continued for 15 days in order to secure the sows from re-infection from the environment during the critical period of C&D, including sow washing and movement to cleaned zones within the sow compartment. Further follow-up during the next months, combined with a continued high level of internal biosecurity to prevent re-infection from the nursery or fattening compartments, confirmed the successful eradication (> 6 months free of *B. hyodysenteriae* in fecal samples). In conclusion, a novel zinc chelate has been succesfully applied for *B. hyodysenteriae* eradication in a closed swine herd infected with a highly Ab- resistant *B. hyodysenteriae* strain.

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Performance and antibiotic use of piglets vaccinated with an *E. coli* F4/F18 vaccination for the prevention of F18-ETEC post-weaning diarrhea

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Introduction

Post-weaning *Escherichia coli* (*E. coli*) diarrhea (PWD), also called post-weaning enteric colibacillosis, remains a major cause of economic losses for the pig industry (1,2). PWD typically causes mild to severe watery diarrhea between 5 and 10 days after weaning and is caused primarily by enterotoxigenic *Escherichia coli* (ETEC). The most common adhesins found on ETEC from PWD in pigs are associated with fimbriae F4 (previously called K88) and F18, while the predominant enterotoxins are heat-labile toxin (LT), heat-stable toxin a (STa), and heat-stable toxin b (STb). In addition to F4 and F18, other fimbrial adhesins, such as F5 (K99), F6 (987P), and F7 (F41), have been associated with PWD, but less frequently (3-6).Therapy to combat PWD typically consists of antibiotic treatment in combination with high doses of ZnO (3000 ppm). Recently, an oral live bivalent *E. coli* F4/F18 vaccine (Coliprotec® F4/F18; Elanco) has become available on the European market, which reduces the impact of PWD provoked by F4-ETEC and F18-ETEC. The objective was to compare technical results of *E. coli* F4/F18 vaccination with previous standard therapeutic approach under field conditions.

Materials and Methods

A 1100-sow farm (weaning at 21 days) with diagnosed problems of PWD due to F18-ETEC was selected for the field evaluation. Piglets were vaccinated through water bowl administration at 18 days with the oral live bivalent *E. coli* F4/ F18 vaccine. At weaning, no standard group medication (ZnO and antibiotics) was applied for prevention of PWD. Piglets were fed a commercial dry feed. Several performance parameters were collected: weight at d0-47, average daily weight gain (ADWG), feed intake (FI), feed conversion rate (FCR), antibiotic use (TI_{100}), mortality and total number and percentage of marketed piglets. Results were analysed using JMP 14.0.

Results

Results are given in Table 1. Oral *E. coli* F4/F18 vaccination pre-weaning reduced the mortality rate. Standard treatment for PWD using colistin could be entirely omitted, resulting in a significantly reduced antibiotic use (TI_{100}). All other piglet performance parameters (ADG, FI and FCR) were at the same level compared to pre-vaccination. Overall, a substantial higher number and percentage of piglets could be marketed at the end of the post-weaning phase.

Table 1. Technical results of a comparative trial using an oral live bivalent *E. coli* F4/F18 vaccination or standard therapeutic approach. Significant differences ($P < 0.05$) are indicated with different superscript

Technical parameter	Control	*E. coli* vaccine
# piglets	12839	10140
Weaning weight	5.27 [a]	5.04 [b]
End weight	22.79	23.23
FCR	1.494	1.469
ADWG (g/d)	362	378
FI (g/d)	540	556
days in nursery (d)	47.5	47.6
Mortality (%)	3.56 [a]	1.67 [b]
# marketed pigs (%)	12382 (96.44%)	9970 (98.32%)
TI_{100}	10 [a]	0 [b]

Conclusions and Discussion

The results show that live *E. coli* F4/F18 vaccination against PWD has similar technical performance parameters, in combination with a significant reduction in the mortality and medication use. In conclusion, control of PWD through *E. coli* F4/F18 vaccination is a good option in order to prevent piglets from the negative clinical outcomes of F18-ETEC infection during the post-weaning period.

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Key words
Escherichia coli F18 – Coliprotec® F4/F18 – post-weaning diarrhea – antibiotic reduction.

Treatment of end-of-nursery piglets with a novel zinc chelate results in longer time-to-treatment in the fattening unit

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Introduction

Swine dysentery caused by *Brachyspira hyodysenteriae* (*B. hyodysenteriae*) is an important intestinal disease with clinical signs typically consisting of muco-haemorrhagic diarrhea. Economic losses are due to mortality, diminished growth rates, deterioration of feed conversion and cost of medical treatment. Diagnosis is performed using pooled faecal samples (microbial culture, PCR test). A novel non-antibiotic zinc chelate has been reported to demonstrate positive effects on fecal quality and consistency, general clinical signs, average daily weight gain and *B. hyodysenteriae* excretion after a 6-day oral treatment (1,2). The objective was to study the time-to-treatment following introduction of 'clean' piglets treated with a zinc chelate (IntraDysovinol® 499 mg/ml; Intracare BV) in cleaned and disinfected compartments of a fattening unit with a history of swine dysentery in the Netherlands.

Materials and Methods

A fattening unit with history of consistent clinical signs of swine dysentery was included in the study. All piglets originated from a *B. hyodysenteriae*-infected breeding herd. In order to eliminate swine dysentery from the fattening unit, a treatment strategy using a novel zinc chelate combined with the Intra cleaning and disinfection protocol (Intracare BV; Netherlands) was designed. Piglets were treated with the zinc chelate for 6 days before transport to the fattening facilities and were housed in freshly cleaned compartments. Strict internal biosecurity measures were implemented to separate piglets introduced after the cleaning protocol from older fattening pigs still potentially infected with *B. hyodysenteriae.*

Results

Until now, 16 weekly batches of piglets have been transported to the fattening unit. Intensive clinical follow-up has revealed no clinical signs of swine dysentery in any of these newly introduced batches. Currently time-to-treatment interval is at least 16 weeks. Approximately 80% of all compartments in the house (Figure 1) are already stocked with piglets treated with the novel zinc chelate prior to introduction in the fattening unit.

Conclusions and Discussion

Zinc chelate treatment of *B. hyodysenteriae*-infected piglets prior to transfer to the fattening unit resulted in an extended time-to-treatment interval.

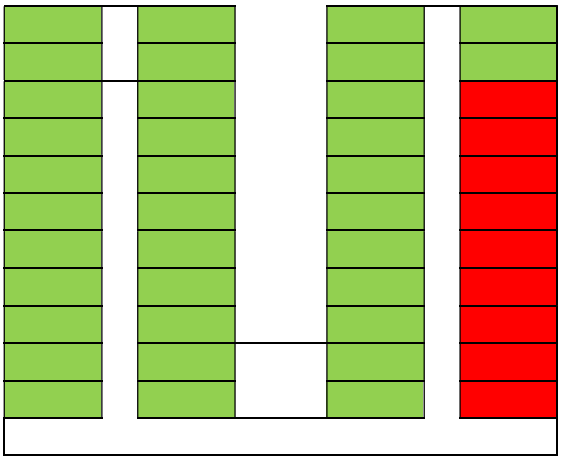

Figure 1. Schematic floor plan of *B. hyodysenteriae*-infected fattening unit. Green compartments are stocked with zinc chelate-treated piglets, red compartments house potentially *B. hyodysenteriae*-infected fattening pigs

Zinc chelate is a novel non-antibiotic treatment for swine dysentery due to *B. hyodysenteriae* that reduced the excretion of *B. hyodysenteriae* following a 6-day oral treatment. Treatment resulted in a lower bacterial load and an extended time-to-treatment interval, mainly in combination with cleaning and disinfection prior to restocking and increased internal biosecurity measures to omit potential spread of *B. hyodysenteriae* from the infected to the non-infected compartments.

References

1. Lammers G. et al., 2019. Treatment of clinical *Brachyspira hyodysenteriae* with zinc chelate in pigs – a blinded, randomised controlled trial. Vet. Rec. 185, 659-665.
2. Vangroenweghe F. et al., 2020. Evaluation of a zinc chelate on clinical swine dysentery under field conditions. Porcine Health Management, 6:1-10.

Key words

Brachyspira hyodysenteriae – zinc chelate – field evaluation – swine dysentery.

Increased performance and reduced antimicrobial use following the treatment of clinical swine dysentery with a novel zinc chelate in an AI/AO fattening unit in the Netherlands

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Introduction

Swine dysentery caused by *Brachyspira hyodysenteriae* (*B. hyodysenteriae*) is an important intestinal disease with clinical signs typically consisting of muco-haemorrhagic diarrhea. Economic losses are due to mortality, diminished growth rates, deterioration of feed conversion and cost of medical treatment. Diagnosis is performed using pooled faecal samples (microbial culture, PCR test). A novel non-antibiotic zinc chelate has been reported to demonstrate positive effects on fecal quality and consistency, general clinical signs, average daily weight gain and *B. hyodysenteriae* excretion after a 6-day oral treatment (1,2). The objective was to compare performance parameters and antimicrobial use between a standard protocol (tiamulin, Denagard™; Elanco) and a zinc chelate (Intra Dysovinol® 499 mg/ml; Intracare BV) on naturally occurring swine dysentery due to *B. hyodysenteriae* in a fattening unit in the Netherlands.

Materials and Methods

An all-in/all-out fattening unit with 800 fatteners, housed in 8 pen of 100 animals was enrolled in the study. The fattening unit had a consistent history of swine dysentery with typical clinical signs of loose stools with addition of blood, mucus and necrotic material. Two subsequent batches of pigs were compared in performance parameters (average daily gain, days in fattening, mortality) and antimicrobial use (TI_{100}). The standard therapeutic approach consisted of individual injections with tiamulin (Denagard™; Elanco) or water medication with tiamulin (Denagard™; Elanco) for 5 consecutive days, depending on the percentage of *B. hyodysenteriae*-affected animals in the pen. The alternative therapy was administration of a novel zinc chelate (Intra Dysovinol® 499 mg/ml) through water medication for 6 consecutive days. Statistical analysis was performed using JMP 14.0.

Results

The results are shown in Table 1. Although *B. hyodysenteriae* infection pressure was fairly high in the fattening unit, average daily gain in the zinc chelate-treated batch was significantly higher as compared to the standard therapeutic approach using tiamulin. Pigs treated with the zinc chelate product had 6 days less in fattening and had a 47% reduction in mortality. The TI_{100} – a parameter to indicate the number of treatments per 100 animals present – was numerically lower in the zinc chelate-treated pigs as compared to pigs treated with the standard therapeutic approach.

Table 1. Performance parameters and antibiotic use (TI_{100}) in a *B. hyodysenteriae*-infected fattening unit treated with a standard therapeutic approach or a novel zinc chelate product

	Tiamulin	Zinc chelate	*P*
Average daily weight gain (g/d)	711	735	< 0.05
Days in fattening (d)	154	148	N/A
Mortality (%)	6.6	3.5	< 0.05
TI_{100}	11.2	9.5	> 0.05

Conclusions and Discussion

Zinc chelate – a novel non-antibiotic treatment for swine dysentery – has been demonstrated to improve general clinical signs and fecal score combined with a reduced excretion of *B. hyodysenteriae* under field conditions (1,2). In the present study, oral treatment with zinc chelate resulted in a higher average daily weight gain in combination with lower mortality and a lower number of days in fattening. Moreover, the TI_{100} was numerically reduced, implying a markedly lower use of antimicrobials to keep clinical signs of swine dysentery under control. In conclusion, the novel zinc chelate was able to improve technical parameters – average daily weight gain, mortality and days in fattening - in combination with a lower use of antibiotics.

References

1. Lammers G. et al., 2019. Treatment of clinical *Brachyspira hyodysenteriae* with zinc chelate in pigs – a blinded, randomised controlled trial. Vet. Rec. 185, 659-665.
2. Vangroenweghe F. et al., 2020. Evaluation of a zinc chelate on clinical swine dysentery under field conditions. Porcine Health Management, 6:1-10.

Key words

Brachyspira hyodysenteriae - zinc chelate - field evaluation – swine dysentery.

Importance of continuous application of a novel zinc chelate for the treatment of clinical swine dysentery to reduce pathogen excretion

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Introduction

Swine dysentery caused by *Brachyspira hyodysenteriae (B. hyodysenteriae*) is an important intestinal disease with clinical signs typically consisting of muco-haemorrhagic diarrhea. Economic losses are due to mortality, diminished growth rates, deterioration of feed conversion and cost of medical treatment. Diagnosis is performed using pooled faecal samples (microbial culture, PCR test). A novel non-antibiotic zinc chelate demonstrated positive effects on fecal quality and consistency, general clinical signs, average daily weight gain and *B. hyodysenteriae* excretion after a 6-day oral treatment (1,2). In practice, treatment regime according to SPC is not always followed, which might impact the clinical results following treatment. Therefore, this study compared pathogen excretion (analysed by PCR) between treatment protocols according to SPC (24/24h, 6-day treatment) and suboptimal (discontinuous) treatment as occasionally performed under field conditions (due to dosing limitations) with a zinc chelate (Intra Dysovinol® 499 mg/ml; Intracare BV) in the treatment of clinical swine dysentery.

Materials and Methods

Several fattening units from one veterinary practice were targeted for the field study. Product application was performed according to SPC (continuous; 24/24h; n = 5 farms) or off-label (discontinuous, 12/24h; due to alternative dosing by farmer; n = 11 farms) for a 6-day treatment period. Following the end of treatment, fecal samples were collected to check for presence of *B. hyodysenteriae* by PCR. Results were reported as positive or negative, with the cut-off at a detection level of 18 cfu/g feces.

Results

In total, 16 farms were identified for sampling. At the end of the treatment period, a total of 50 samples were collected, 14 samples in farms (n = 5) that treated the animals according to SPC specifications and 36 samples in farms (n = 11) where fatteners were treated off-label due to miscommunications between farmer and responsible vet or due to treatment limitations related to the dosing equipment. Results are given in Figure 1. In the 5 farms that treated according to SPC, only 1 out of 14 fecal samples (7.1%) was positive for *B. hyodysenteriae*, whereas in the farms were fatteners were treated off-label, 13 out of 36 samples (36.1%) were positive for *B. hyodysenteriae*.

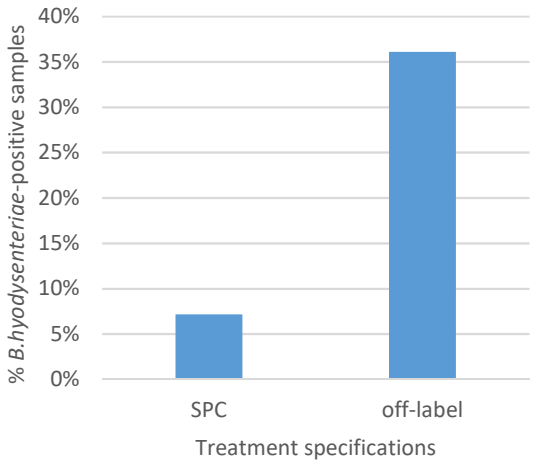

Figure 1. Percentage of *B. hyodysenteriae*-positive samples following correct (SPC) or incorrect (off-label) treatment with a novel zinc chelate

Conclusions and Discussion

Zinc chelate (Intra Dysovinol® 499 mg/ml) is a novel non-antibiotic treatment for swine dysentery resulting in a decreased excretion of *B. hyodysenteriae* following treatment according to SPC specifications (continuous; 24/24h for a 6-day treatment period). Discontinuous application (off-label and not according to the SPC) does not results in the same reduction of *B. hyodysenteriae* excretion following a 6-day oral treatment. In conclusion, correct application according to SPC of the novel zinc chelate for treatment of swine dysentery is crucial to obtain clear reduction in *B. hyodysenteriae* excretion.

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1. Lammers G. et al., 2019. Treatment of clinical *Brachyspira hyodysenteriae* with zinc chelate in pigs – a blinded, randomised controlled trial. Vet. Rec. 185, 659-665.
2. Vangroenweghe F. et al., 2020. Evaluation of a zinc chelate on clinical swine dysentery under field conditions. Porcine Health Management, 6:1-10.

Key Words

Brachyspira hyodysenteriae – swine dysentery – fattening – SPC – zinc chelate.

Detection of *Mycoplasma hyopneumoniae* in coughing pigs under field conditions in different diagnostic sample types

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Introduction

Mycoplasma hyopneumoniae (*M. hyopneumoniae)*, the primary pathogen of Enzootic Pneumonia, occurs worldwide and causes major economic losses to the pig industry. The pathogen adheres to and damages the ciliated epithelium of the respiratory tract. Affected pigs show chronic coughing, are more susceptible to other respiratory infections and have a reduced performance (1). Piglets become infected with *M. hyopneumoniae* during the suckling period and have been shown positive from weaning onwards (2-6). Moreover, once infected with *M. hyopneumoniae*, animals can excrete the pathogen over a long period of time, with total clearance lasting for over 254 days post-infection (7). Recent research has shown that in pigs experimentally infected with *M. hyopneumoniae,* the pathogen recovery by different diagnostic techniques is quite variable (8). Early *M. hyopneumoniae* detection was possible through laryngeal swabs, whereas nasal swabs and oral fluids (OF) were only to a lesser extent positive and in a later stage of the disease. The objective of the current study was to analyse *M. hyopneumoniae* detection from field samples (lung tissue, OF or trachea-bronchial swabs (TBS)) collected on farms with clinical respiratory problems - associated with coughing - in Belgium and the Netherlands by swine practitioners.

Materials and Methods

The study was performed from January 2018 till December 2018. Diagnostic samples for detection of *M. hyopneumoniae* were collected in clinically coughing pigs of different ages at 286 swine farms by 52 different swine practitioners throughout Belgium and the Netherlands. Depending on the field situation, one of the three different sample types, *i.e.* TBS, lung tissue or OF, was collected by the swine practitioner. Samples were submitted to a commercial veterinary diagnostic laboratory (IVD GmbH) for analysis on presence of *M. hyopneumoniae*. Samples were analysed using a PCR test and results were reported as positive/negative.

Results

The main findings of the comparative study are shown in Table 1. Overall, *M. hyopneumoniae* detection was at least 3 times higher in TBS samples as compared to OF. Lung tissue samples from pathological lesions were 50% more reliable to detect *M. hyopneumoniae* as compared to OF.

Conclusions and Discussion

The current study was not a direct comparison between the three diagnostic sampling techniques. However, all samples were collected in pigs showing respiratory distress and coughing. The decision of the specific sampling technique to be used was made by swine practitioner present at the farm. However, due to the large number of samples submitted from each sample type, some indirect conclusions can be postulated.

Table 1. Different diagnostic sample types with the percentage of positive samples and the total number of samples collected per sample type

Diagnostic sample type	% positive samples	# samples collected
Oral fluids	12	42
Lung tissue	18	39
Tracheo-bronchial swabs	38	205

The results were in line with an earlier experimental study (8) demonstrating that sensitivity of OF was lower than other deeper respiratory tract sampling techniques, such as laryngeal swabs or TBS. Moreover in this study, OF were positive later in the pathogenesis of *M. hyopneumoniae*, which implies a later detection of the circulating infection. In conclusion, diagnosis of *M. hyopneumoniae*-related respiratory problems is more appropriately conducted using TBS, which has a higher certainty to detect the pathogen as compared to OF.

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Key words

Mycoplasma hyopneumoniae – diagnostics – oral fluids – lung tissue – trachea-bronchial swabs.

Evaluation of a novel zinc chelate in the treatment of clinical swine dysentery under field conditions in the Netherlands

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Introduction

Brachyspira hyodysenteriae is the primary cause of swine dysentery and primarily affects pigs during the grow/finishing stage. Economic losses are due to mortality, diminished growth rates, deterioration of feed conversion and cost of medical treatment. Diagnosis is performed using pooled faecal samples (microbial culture, PCR test). A novel non-antibiotic zinc chelate has been reported to demonstrate positive effects on fecal quality and consistency, general clinical signs, average daily weight gain and *B. hyodysenteriae* excretion after a 6-day oral treatment (1). The objective was to evaluate the zinc chelate (Intra Dysovinol® 499 mg/ml; Intracare BV) on naturally occurring swine dysentery due to *B. hyodysenteriae* under Dutch field conditions.

Materials and Methods

Two conventional pig farms with clinical signs of swine dysentery were selected and pigs were randomly attributed to control/treatment. Pigs were treated with the oral zinc chelate according to label instructions. Several individual clinical (general clinical signs, total fecal score, antibiotic treatment, mortality) and performance parameters (average daily weight gain (ADWG)) were collected besides individual fecal samples were collected for qPCR analysis of *B. hyodysenteriae* at sample days (SD) 0, 2, 4, 6 and 14. Statistical analysis was performed using JMP 14.0.

Results

Oral administration of zinc chelate resulted in improvement of general clinical signs (90.0% *vs.* 73.6% animals with normal clinical score at SD6) and total fecal score (0.39 *vs.* 1.23 at SD14; Figure 1). The zinc chelate-treated pigs had a significantly higher ADGW throughout the entire study as compared to the control pigs. During the treatment period (SD0-6), treated pigs grew 825 g/d, whereas the control pigs grew 619 g/d. In the post-treatment period (SD6-14), treated pigs continued to grow significantly (903 g/d) as compared to control pigs (505 g/d). Excretion of *B. hyodysenteriae* decreased in the treated pigs from SD2 onwards and remained low until SD6. No additional antimicrobial treatments were needed in the zinc chelate-treated group, whereas 35 percent of the pigs in the control group were treated with an antibiotic at least once. No mortality occurred in both groups.

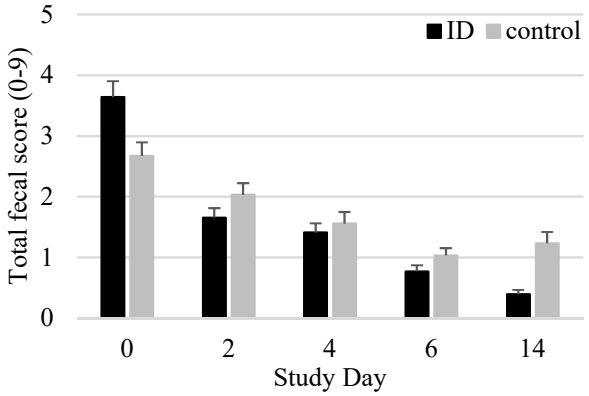

Figure 1. Total fecal score of zinc chelate-treated (ID) and control pigs per study day from SD 0 to 14. Pigs were treated with zinc chelate from SD0-6.

Conclusions and Discussion

Zinc chelate is a novel non-antibiotic treatment for swine dysentery that reduces *B. hyodysenteriae* shedding within its 6-day treatment, improving clinical signs and fecal quality within 2-4 days of administration in pigs naturally infected with *B. hyodysenteriae* (1,2). The treatment resulted in a higher growth rate during and after the treatment and improved general health. In the zinc chelate-treated pigs, no mortality was observed and no additional therapeutic treatments were necessary in contrast to the control pigs. Positive effect of the zinc chelate treatment remain for at least 8 days after cessation of oral treatment. However, pigs might become reinfected in a highly contamined environment. Therefore, treatment should be combined with additional management practices to reduce environmental infection pressure (2).

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Key words

Brachyspira hyodysenteriae – swine dysentery – zinc chelate – pig performance – fecal score.

Improved performance and decreased mortality in piglets vaccinated with an *E. coli* F4/F18 vaccination for the prevention of F4-ETEC post-weaning diarrhea

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Introduction

Post-weaning *Escherichia coli* (*E. coli*) diarrhea (PWD), also called post-weaning enteric colibacillosis, remains a major cause of economic losses for the pig industry (1,2). PWD typically causes mild to severe watery diarrhea between 5 and 10 days after weaning and is caused primarily by enterotoxigenic *Escherichia coli* (ETEC). The most common adhesins found on ETEC from PWD in pigs are associated with fimbriae F4 (previously called K88) and F18, while the predominant enterotoxins are heat-labile toxin (LT), heat-stable toxin a (STa), and heat-stable toxin b (STb). In addition to F4 and F18, other fimbrial adhesins, such as F5 (K99), F6 (987P), and F7 (F41), have been associated with PWD, but less frequently (3-6). Therapy to combat PWD typically consists of antibiotic treatment. Recently, an oral live bivalent *E. coli* F4/F18 vaccine (Coliprotec® F4/F18; Elanco) has become available on the European market, which reduces the impact of PWD provoked by F4-ETEC and F18-ETEC. The objective was to compare technical results of *E. coli* F4/F18 vaccination with previous standard therapeutic approach under field conditions.

Materials and Methods

A sow farm (weaning at 25 days) with diagnosed problems of PWD due to F4-ETEC was selected for the field evaluation. Piglets were vaccinated through water bowl administration at 18 days with the oral live bivalent *E. coli* F4/ F18 vaccine. At weaning, no standard group medication with antibiotics was applied for prevention of PWD. Piglets were fed a farm-mixed liquid feed formulation. Several performance parameters were collected: weight at d0-47, average daily weight gain (ADWG), feed intake (FI), feed conversion rate (FCR), antibiotic use (TI_{100}) and mortality. Results were analysed using JMP 14.0.

Results

Results are given in Table 1. Oral *E. coli* F4/F18 vaccination pre-weaning reduced the mortality rate. Standard treatment for PWD using colistin could be entirely omitted, resulting in a significantly reduced antibiotic use (TI_{100}). Several piglet performance parameters (ADG, FCR and mortality) significantly ($P < 0.05$) improved as compared to pre-vaccination levels.

Table 1. Technical results of a comparative trial using an oral live bivalent *E. coli* F4/F18 vaccination or standard therapeutic approach. Significant differences ($P < 0.05$) are indicated with different superscript.

Technical parameter	Control	*E. coli* vaccine
# piglets	1840	1910
Weaning weight	7.00	6.98
End weight	21.50 [a]	23.42 [b]
FCR	1.88	1.83
ADWG (g/d)	358 [a]	420 [b]
days in nursery (d)	40	40
Mortality (%)	3.31 [a]	2.30 [b]
TI_{100}	6.3 [a]	0 [b]

Conclusions and Discussion

The results show that live *E. coli* F4/F18 vaccination against PWD due to F4-ETEC results in improved technical performance parameters. Average daily weight gain and weight at end of nursery were significantly better, resulting in heavier to be transferred to fattening. Moreover, this was combined with a significant reduction in mortality and medication use. In conclusion, control of PWD through *E. coli* F4/F18 vaccination is a good option in order to prevent piglets from the negative clinical outcomes of F4-ETEC infection during the post-weaning period and leads to consistently improved economical results in the batches of vaccinated piglets.

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Key words

Escherichia coli F4 – Coliprotec® F4/F18 – post-weaning diarrhea – antibiotic reduction – improved performances.

Distribution study of *Brachyspira hyodysenteriae* medication in liquid feeding systems

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Introduction

Liquid feed may be prepared from simply mixing dry rations with water, however, the main advantage of liquid feeding is the ability to use other raw materials such as by-products from the food industry (*e.g.* cheese whey, liquid wheat starch, potato steam peel). This opportunity is economically interesting and results in higher feeding flexibility and increased appreciation towards circular food chains. Central computer-controlled automated systems are typically used for liquid feed preparations. Following thorough mixing, these preparations are transported to the pig compartments, where they are directly consumed.

Intra Dysovinol® (ID; Intracare BV Veghel, The Netherlands) is a solution for use in drinking water for the treatment and metaphylaxis of swine dysentery in pigs. However, an increasing number of pigs receives liquid feed without additional drinking water. Therefore, we investigated the homogeneity following addition of the product to liquid feed of different compositions.

Materials and Methods

Tests were performed on two commercial farrow-to-finish farms that used computer-controlled automated mixing systems equipped with cube-shaped tanks (farm A) or round tanks (farm B). Farm A mixed dry rations with water, whereas farm B prepared different combinations of dry rations with multiple by-products. Both farms prepared specific recipes for sows, piglets and fattening pigs.

All formulations contained a dry weight of approximately 25%. Animals received no additional drinking water. Therefore, product dosage was calculated for a 75% water content of the prepared liquid feed. Experiments were performed with 0.4 ml per liter water resulting in a 0.3 ml addition of ID per per kg liquid feed.

Results

The calculated amount of ID was added to the mixing tank and mixed for 2 minutes. After mixing, a random sample was taken from the mixing tank. The product content was measured and compared to the expected amount at least 24 hours after preparation (Table 1).

Table 1. Details of the different liquid feed compositions and recovery of the product.

Farm	Recipe	TV (kg)	IDc	IDm	Recovery
A	Sows	940	0.32	0.30	94%
A	Piglets	940	0.29	0.30	103%
B	Piglets	980	0.30	0.26	87%
B	Sows	1770	0.30	0.26	87%
B	Fattening	1860	0.30	0.32	107%

TV - total volume expressed in kg; ID - Intra Dysovinol®; c - calculated; m - measured

Conclusions and Discussion

Addition of the product to common automated liquid feed systems resulted in 87-107% recovery after 2 minutes of mixing in different liquid feed formulations. From an analytical point of view, liquid feed is a complex organic matrix, negatively affecting assay accuracy. Therefore, the observed maximum off-target deviation of 13% (87% recovery) falls within the expected analytical variation.

Liquid feed preparation generally starts with the water component and medicinal additions are usually added at this stage to promote dissolution. However, for the current study ID was added after the addition of dry and raw materials to the water, since a background sample of the complete composition without the product was required. Therefore, the presented results reflect a worst-case scenario. In addition, samples were measured after at least 24 hours, while the liquid feed is typically consumed by the pigs within an hour after preparation.

In conclusion, the addition of ID to different types of liquid feed formulations in volumes of 940 to 1940 kg resulted in a homogenous distribution of the product throughout the entire liquid feed after only 2 minutes of mixing. The current results indicate all animals will receive the same calculated amount of product for accurate treatment using the liquid feed application.

Acknowledgments

Pig farmers are greatly acknowledged for their contribution in using their liquid feeding equipment.

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Oral vaccination with a live bivalent *E. coli* vaccine improved growth and feed conversion ratio in different farms with post-weaning diarrhea due to F4-ETEC strains

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Introduction

Post-weaning diarrhea (PWD) inflicts major economic losses on the pig industry and typically develops between 5 and 10 days post-weaning (1,2). The primary cause of PWD is enterotoxigenic *E. coli* (ETEC), a pathotype characterized by the expression of F4 and F18 fimbriae as well as the production of enterotoxins, such as LT, STa and STb (1). An oral live *E. coli* F4/F18 vaccine (Coliprotec® F4/F18; Elanco) is now available for the active immunization of pigs against PWD (2). This study assessed the effect of Coliprotec® F4/F18 vaccination on the performance of pigs from five different farms during the entire fattening period.

Materials and Methods

This study was conducted on five different British indoor wean-to-finish farms (Farms 1-5). The farms received piglets from four different outdoor breeding sites that carried F4-ETEC strains, resulting in chronic PWD issues.

All piglets were weaned at four weeks of age. In each wean-to-finish farm, two consecutive batches of piglets were enrolled in the same sequence: one non-vaccinated (NV) and one vaccinated (V). Coliprotec® F4/F18 was administered through drinking water, using either a pump-dosing system (Farm 1) or manual application in the water troughs (Farms 2 to 5). Piglets from Farm 1 were vaccinated on-site (three days post-weaning), whereas piglets from Farms 2 to 5 were vaccinated at the breeding sites (between the 18th day of age and weaning). For each batch, average weaning weights, eviscerated carcass weights (ECW) and feed conversion ratio (FCR) were recorded.

The ECW data were analyzed with ANOVA (SPSS® V.23.0, Illinois, USA).

Results

The V batches in Farms 3 and 5 had significantly higher ECW than the NV batches ($P < 0.05$), whereas in Farms 1 and 4 the V batches had only numerically higher ECW than the NV batches ($P > 0.05$). In Farm 2 the V batch had a numerically lower ECW than the NV batch ($P > 0.05$).

In all farms except Farm 2, the V batches exhibited a higher weight gain between weaning and slaughter compared to the NV batches (Table 1).

In all farms except Farm 2, higher average daily weight gain (ADWG) was observed in the V batches as compared to the NV batches (Table 2). The V batches in Farms 1, 3 and 4 exhibited reduced FCR when compared to the NV batches, whereas an increase in FCR were detected between the NV and the V batches in Farms 2 and 5 (Table 3).

Table 1. Differences (in kg) between the average weaning weights and the average slaughter weights in NV (control) and V (*E. coli* vaccinated) batches of the different farms.

	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5
NV	106.40	102.10	100.40	113.88	107.82
V	107.39	99.74	105.80	114.69	113.73

Table 2. ADWG (g/pig/day) between NV (control) and the V (*E. coli* vaccinated) batches of the different farms.

	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5
NV	793.21	848.62	836.29	881.62	807.93
V	828.77	787.29	916.16	922.64	825.30

Table 3. FCR between NV (control) and V (*E. coli* vaccinated) batches of the different farms.

	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5
NV	2.26	2.33	2.11	2.29	2.31
V	2.42	2.21	2.35	2.36	2.30

Conclusions and Discussion

In four out of five farms included in this study, oral *E. coli* vaccination of piglets suffering PWD resulted in better growth performances during the fattening period. Additionally, in three out five farms the V batches had an improved FCR as compared to the NV batches. Overall, oral *E. coli* vaccination could be an effective option to reduce the negative impact of PWD due to F4-ETEC on profitability of integrated systems.

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Non-antimicrobial prophylactics increase intestinal mucosa survival following enteric bacteria challenge *in vitro*

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Introduction

Increase in multi-drug resistant bacterial infections have led to restrictions on antibiotics available to treat swine. In-feed delivery of non-antibiotic prophylactics support beneficial bacterial communities while helping maintain epithelial lining integrity. Together, these properties may help mitigate intestinal lesions associated with bacterial infections. Therefore, this study aimed to evaluate the *in vitro* efficacy of compound F, a fungal fermented rye and compound P, a blend of short and medium chain fatty acids, including coated butyrate) to mitigate lesions associated with colon exposure to *Brachyspira hyodysenteriae, Lawsonia intracellularis* or *Salmonella* Typhimurium.

Materials and Methods

For each pathogen, 5 pigs free of gastrointestinal clinical signs (5-6 weeks of age) were used as tissue donors (n=6 colon explants/pig). From each pig, segments of colon were aseptically collected following euthanasia. Next colon serosa was separated from the mucosa and explants (4 cm^2) were individually cultured at 37ºC. Explants were treated in culture with compounds F and P (using suggested *in vivo* concentrations), and exposed to one of the following inocula combinations: 1) pathogen only; 2) compound only; 3) pathogen and compound together. Explants from each group (n=5 colon explants/inoculum group/pathogen) were fixed in formalin after 8 hours of exposure to *B. hyodysenteriae and L. intracellularis* and 2 hours to *S.* Typhimurium. Haematoxylin and eosin stained sections were evaluated according to the percentage of healthy epithelium covering the mucosal aspect of explants. Generalized estimating equations, clustering by pig, was used to compare the percentage of healthy epithelium between groups.

Results

Explants treated with compound F had significantly higher levels of epithelial coverage following exposure to *L. intracellularis*, when compared to non-treated, challenged explants (Fig. 1B). Although not statistically significant, explants treated with compound F and challenged with *S.* Typhimurium also had higher average epithelial coverage than untreated explants challenged with the same pathogen (Fig. 1C). In contrast, explant exposure to *B. hyodysenteriae* and compound F did not result in increased epithelial coverage, when compared to the pathogen-exposed group (Fig. 1A). Compound P treatment did not significantly increase epithelial coverage upon to all pathogens challenge, when compared to explants inoculated with each pathogen alone.

Conclusions and Discussion

Compound F treatment induced higher epithelial survival *in vitro* when explants were challenged with *L. intracellularis* and *S.* Typhimurium. This suggests a potential prophylactic effect. The same was not true for compound P, likely due to the incomplete digestion of the anti-acid delivery coat used. It is important to recognize the difference in virulence mechanism of the three bacteria. Thus, it is hypothesized that epithelial coverage did not reflect compound effectiveness in preventing tissue damage following *B. hyodysenteriae* infection, since this pathogen does not induce epithelium death directly. Other analyses are underway to investigate the mode of action of each compound, and to further validate the infection models.

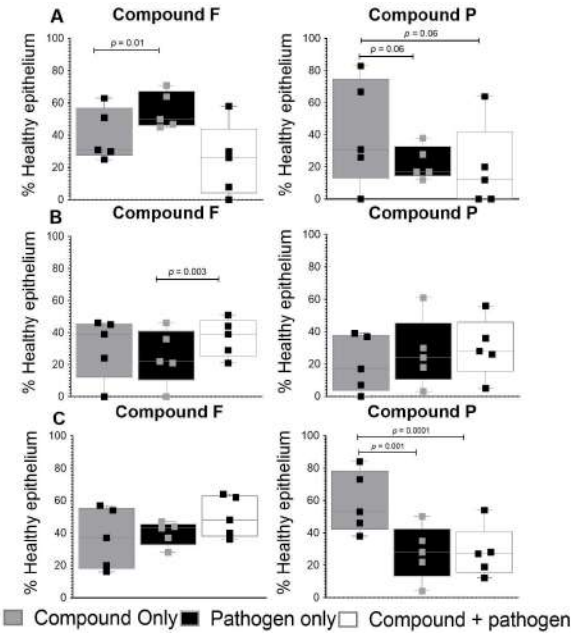

Figure 1. Percentage of explant epithelium coverage. A) *B. hyodysenteriae;* B) *L. intracellularis;* C) *S.* Typhimurium. Higher and lower bars represent the standard deviation. Central bar depicts the mean. Whiskers represent the range of values. Blocks represent data from each individual pig.

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Replication of clinical *Streptococcus equi* subspecies *zooepidemicus* disease in sows

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Introduction

Streptococcus equi subspecies *zooepidemicus* (*S. zooepidemicus*) is a commensal bacteria of horses that causes opportunistic infections in horses and other mammals, including humans (1, 2). *S. zooepidemicus* has also been previously reported to cause severe disease in pigs, with a large Chinese outbreak in 1975 reporting mortality reaching 300,000 animals (3). Reports of disease in the United States are infrequent and few cases have been reported by the Iowa State University Veterinary Diagnostic Laboratory over the last 10 years (4); however, in 2019, high mortality events in cull sows and feeder pigs in assembly yards in the U.S. and Canada were attributed to *S. zooepidemicus* (4). Isolates from the recent mortality events show a high degree of genetic similarity to each other and the isolate associated with the Chinese outbreak in 1975 (5). To better understand the progression of disease and develop a model to replicate disease for future work, the present study assessed the susceptibility of sows to a mortality event isolate of *S. zooepidemicus* and compared that with a genetically distinct equine *S. zooepidemicus* isolate.

Materials and Methods

Isolates screened were from the swine outbreak in TN or a horse with pneumonia and a pleural abscess. Five sows each were challenged with 5mL of ~10^8 colony forming units/mL of each isolate of *S. zooepidemicus* oronasally and monitored for clinical disease. Pigs were euthanized if disease became severe and systemic distribution of *S. zooepidemicus* was determined.

Results

No *S. zooepidemicus* was isolated from sows via tonsil swab prior to challenge. Animals challenged with the swine *S. zooepidemicus* isolate showed clinical signs of vomiting, inappetence, purulent nasal discharge (n=1), severe lethargy, severe depression, and reluctance or inability to rise. Clinical signs began by 24 hours post-infection (hpi) with severe depression and lethargy being noted by 36 hpi. One sow was euthanized at 36 hpi (6082), two sows were found dead at 48 hpi (5456, 6004), and two sows were euthanized at 56 hpi (6093, 6808). Systemic distribution of *S. zooepidemicus* is found in Table 1. Gross lesions at necropsy were minimal with some organ congestion and small areas of consolidation in the lungs of two sows (6082, 6093). One lung of sow 6004 was hemorrhagic and she was found with epistaxis post-mortem.

No illness was noted in the animals challenged with the equine *S. zooepidemicus* isolate and it was cleared from the nasal cavity and tonsil of all animals by 7 days post challenge.

Table 1. Systemic distribution of the swine mortality event *S. zooepidemicus* isolate

	Nasal	Tonsil	Joint	Serosa	CSF[1]	BALF[2]	Serum	Spleen
6082	+	-	0	3	1	2	44	TN[3]
5456	+	-	TN	TN	TN	TN	TN	TN
6004	+	+	84	TN	TN	TN	TN	TN
6093	-	+	0	0	1	0	0	0
6808	+	-	0	0	2	0	0	104

[1]cerebrospinal fluid; [2]bronchoalveolar lavage fluid; [3]too numerous to count

Conclusions and Discussion

Here, we were able to induce disease with a swine isolate of *S. zooepidemicus* in sows and disease presented similarly to what has been seen in the field. Sows were more susceptible to challenge with the *S. zooepidemicus* isolate from the mortality event in TN than a genetically distinct horse isolate. The difference in susceptibility may be associated with the presence of different virulence factors, which will be important to investigate in future work. The challenge model developed here will enable future studies to investigate the importance of different virulence factors to the pathogenicity of this isolate and to assess potential vaccines for prevention of disease.

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Importance of capsular immunity in protection against *Glaesserella parasuis*

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Introduction

Glaesserella (*Haemophilus*) *parasuis* is a Gram negative bacterium in the Pasteurellaceae family. It is the causative agent of Glässer's disease in swine, a disease characterized by fibrinopurulent polyserositis, arthritis, and meningitis, and a bacterial contributor to porcine respiratory disease complex (1). *G. parasuis* is an early colonizer of the swine respiratory tract and a member of the respiratory microbiota of healthy animals. Isolates of *G. parasuis* are characterized by capsular polysaccharide into 15 serovars (SV) through serology or PCR analysis of the capsule locus (2, 3). Capsule is an important virulence factor of *G. parasuis*, though isolate SV does not correlate exactly with disease potential (4, 5). *G. parasuis* control has been challenging in production settings due to the ubiquitous distribution of *G. parasuis*, rapid onset of disease, and the lack of broadly effective vaccines. The difficulty generating broadly effective vaccines is attributed, in part, to strain and serovar specific immunity associated with bacterin vaccination, which results in minimal protection against heterologous isolates (6). To better understand the role of capsular immunity and assess the capacity of protein based immunity to provide protection against challenge, we evaluated an avirulent, capsule-deficient mutant of the SV5 strain HS069 (HS069Δcap) as a whole cell bacterin and compared its efficacy against homologous and heterologous challenge with a bacterin derived from the wild type HS069.

Materials and Methods

To assess the ability of protein based immunity to provide broad protection against *G. parasuis*, pigs were vaccinated twice with HS069Δcap, the wild type HS069, or adjuvant only at a 21 day interval. Serum samples were assessed for antibody titers at days 0, 21, and 42. On day 42 of the study, animals were challenged with the homologous SV5 strain (HS069), or one of the following heterologous strains: 12939 (SV1), 2170B (SV4), Nagasaki (SV5), or MN-H (SV13). Animals were evaluated over a 14 day period for clinical signs of Glässer's disease and euthanized when severe disease presented. Samples were collected to confirm systemic distribution of *G. parasuis*.

Results

Vaccination with HS069Δcap bacterin and the wild type HS069 bacterin generated strong antibody titers to whole cell sonicate of all challenge strains and boost vaccination resulted in an amnestic antibody responses. Animals vaccinated with HS069Δcap were protected against challenge with the homologous strain (HS069) as well as the heterologous strain 12939, 2170B, and MN-H; however, animals challenged with the heterologous SV5 strain Nagasaki showed no improvement in survival, though median survival time was extended. Comparatively, vaccination with the wild type HS069 provided protection against all challenge strains, including Nagasaki.

Conclusions and Discussion

Differences in protection between bacterins generated from HS069Δcap and the wild type HS069 against challenge with Nagasaki indicate the importance of capsular directed immunity to protection against this *G. parasuis* isolate. For the other challenge isolates in this study, capsular immunity was not essential to prevent the development of disease. Further assessment indicated differences in the quantity and distribution of capsular polysaccharide between Nagasaki and HS069, which may have resulted in the differences in protection between the bacterins after challenge with Nagasaki. This provides essential information for *G. parasuis* vaccine development and indicates capsular immunity is only essential for some isolates of *G. parasuis*, while protein directed immunity can provide protection against less encapsulated strains.

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Co-infection of *Mycoplasma hyopneumoniae, Mycoplasma hyorhynis* and *Mycoplasma flocculare* in pneumonia lesions of slaughtered pigs

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Introduction

Respiratory problems, especially in the lower respiratory tract, are economically the most important diseases in intensive pig herds. *Mycoplasma hyopneumoniae (Mhp)* the main etiological agent of porcine enzootic pneumonia, a disease frequently observed in slaughter-age pigs worldwide (1). Little is known about the dynamics of coinfection between *Mhp* and other pneumonic mycoplasma species. Hitherto, it is not clear whether *Mycoplasma hyorhinis (Mhyor)* and *Mycoplasma flocculare (Mfloc)* are capable of enhancing the severity of enzootic pneumonia. This study investigated the presence of *Mhp, Mhr* and *Mfloc* in lung samples of slaughter pigs and aimed at correlating the amount of these pathogens with the extent lung lesions in slaughter pigs.

Materials and Methods

Two hundred right cardiac lobes were collected in a slaughterhouse in São Paulo, from pigs from the Brazilian Southeast states, which were collected at eight different moments (25 lungs/time). The lobes were collected and divided into five groups (n=40)according to the degree of pneumonia (Table 1). The lungs collected were carefully selected so that 40 samples from each group were collected. The other pulmonary lobes of the selected lungs were also evaluated and their scores were recorded for a second analysis. Differences between groups were evaluated by Kruskal-Wallis test ($p<0.05$), and correlation between data was made by Spearman's correlation test ($p<0.05$). DNA quantification of *Mhp, Mhyor* and *Mfloc* in lung tissue was performed by multiplex real time qPCR (3).

Results

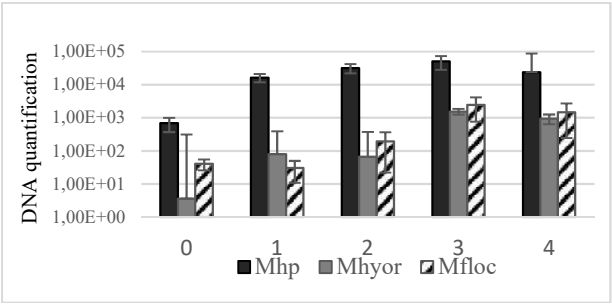
The number of positive samples for each micro-organism for each group is shown in Table 1. Almost all samples were positive for Mhp, whereas less than 25% or 20% were positive for Mhr and Mfloc, respectively.

The correlation between the mean values of quantification of mycoplasmas and the groups of lung lesions scores is shown in Fig 1. There was a significant positive correlation between the lobe score and the *Mhp* quantification (R^2=0.42). For *Mhyor*, the correlation was significant, but low correlation (R^2=0.16). Regarding *Mfloc*, the data presented a significant and negative correlation (R^2=0.18). For all *Mycoplasma* sp. evaluated, there was positive correlation between the DNA quantification and the whole lung lesion score of the entire lung (R^2=0.38; 0.17; 0.2 for *Mhp*, *Mhyor* and *Mfloc*, respectively). There was a significant and negative correlation between the amounts of *Mhp* and *Mfloc* in the lungs , (R^2=0.3). There were no significant associations between *Mhp* and *Mhyor*, nor between *Mhyor* and *Mfloc*.

Table 1. Number of positive samples for each micro-organism in the groups of classification of lung lesion score.

Group	Degree of lung pneumonia (%)	Mhp	Mhr	Mfloc
0 (n=40)	0%	34	4	13
1 (n=40)	1 - 25%	40	5	8
2 (n=40)	26 - 50%	40	18	10
3 (n=40)	51 - 75%	37	9	5
4 (n=40)	76 - 100%	39	9	4

Figure 1. Mean DNA quantification of the three different mycoplasmas in different groups of the right cardiac lobe lesion score.



Conclusions and Discussion

The results showed that the lesions of the entire lung or the right cardiac lobe were moderately and poorly correlated with the bacterial load of *Mhp and Mhyor,* respectively. The importance of the negative correlation between the amount of Mhp and Mfloc warrants further research.

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Seroprevalence of *Lawsonia intracellularis* in selected pig farms in Malaysia

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Introduction

Ileitis is also known as porcine proliferative enteropathy (PPE) is a common disease worldwide caused by *Lawsonia intracellularis* (LI). It exists in 3 forms: acute, chronic or subclinical and has been proven that even in subclinical form, it can reduce average daily weight gain (ADG) by 37-42%, and increase the feed conversion ratio (FCR) by 27-37%, causing a negative impact of at least €3 per affected growing pig (5). Farms with poor feed conversion also showed significantly higher ileitis ELISA values in the fattening period (3.). In this study we aim to determine the herd, animal and age group seroprevalence in order to monitor the pattern of LI infection in Malaysia.

Materials and Methods

In 2019, a total of 600 serum samples (n=600) were collected from 12 commercial pig farms in different areas in Malaysia in this cross-sectional study. 50 samples were collected per farm as stated in Table 1. All samples were tested with Svanovir® Ileitis-Ab blocking ELISA test kit to detect specific antibodies against LI according to manufacturer's instructions.

Table 1. Number of blood samples per age group

Age Group	No of sample
Gilts	5
Sows	5
3W-4W (Start of nursery)	5
8W-10W (End of nursery)	10
12W (Fatteners)	10
18W	10
24W (Finishers)	5
Total	50

Results

It was found that the farm seroprevalence was 100% where all the herds had been exposed to *LI* in at least one of the production phase, as previously reported (1,4). The animal seroprevalence was 33% (198/600). As for age group seroprevalence, the average Malaysia seroprofile (Figure 1) showed the highest percentage of positive samples in gilts and sows (60% and 69%). The lowest prevalence was in piglets at 3-4W and 8-10W (9% and 6%). While after nursery, the seroprevalence increases to 18% at 12W, then to 45% and 58% at 18W and 24W respectively.

Conclusions and Discussion

The 100% farm prevalence and the widespread nature of infection observed in this study suggest that the disease is endemic on most of the Malaysian farms especially on the breeder and fattening groups. While most farms in Malaysia are single site farrow-finish farm, many raise their own gilts in the same system with the porkers. Therefore, it creates a vicious circle of exposure where we saw a high seroprevalence of both the breeders and finishers group.

Piglets at the start of nursery (3-4W) had more positive samples than piglets at the end of nursery (8-10W). This is expected where part of these antibodies at the start of nursery are from maternal side, and they will disappear in most cases by 6 weeks.

It was also observed in this study that seroconversion starts typically between end of nursery and 12W. Hence, considering that it takes 2-3 weeks to seroconvert after infection, these pigs were either starting to get infected at the end of nursery or early at the fattening unit.

Comparing with average European seroprofiles (2) (Figure 1), Malaysia farms have lower seroprevalence in all age group. This could be attributed to the higher usage of antibiotics in Malaysia farms such as tiamulin, tylosin and tetracycline which are commonly used to control respiratory and reproductive infection.

This study does not only report the prevalence and the presence of LI infection in Malaysia, it also helps to understand its epidemiology in order to develop an effective monitoring, prevention and control program.

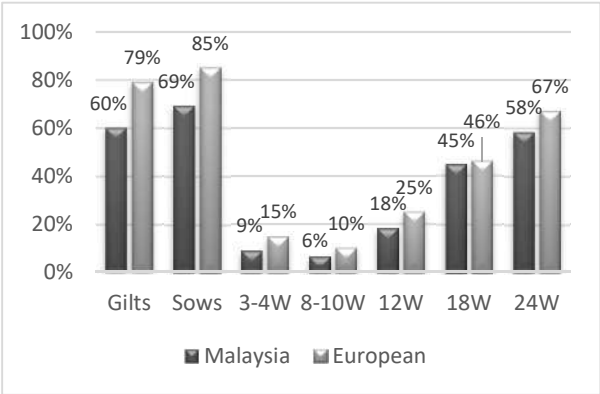

Figure 1. Average Seroprofile in Malaysia vs Europe (12 Malaysian & 342 European farms (15,997 serum samples)

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Occurrence of *Bordetella bronchiseptica* and *Pasteurella multocida* associated with non-progressive atrophic rhinitis on pig farms in Colombia

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Introduction

Atrophic Rhinitis (AR) is an important infectious disease caused by *Bordetella bronchiseptica* (Bb), either alone, in which case it is called Non-Progressive AR (NPAR) or by co-infection with *Pasteurella multocida* (Pm), which is known as Progressive Atrophic Rhinitis (PAR)[1]. The main damage caused by PAR is turbinate atrophy or septum deformation, leading to retarded growth. Slaughterhouse turbinate nasal lesion scoring is one of the main tools used to evaluate the incidence of the disease under field conditions. The aim of this study was to evaluate the presence of Bb and Pm and its correlation with the turbinate nasal lesions seen in the slaughterhouse evaluation on farms located all over Colombia.

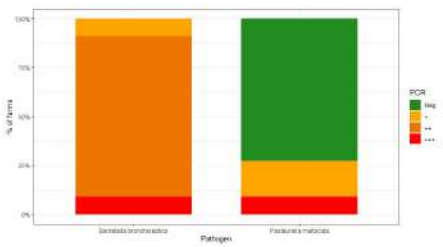
Materials and Methods

A total of 11 swine farms with different farm managements and herd sizes located in Colombia was evaluated. No vaccination against AR was implemented on any of the farms. OF samples from different age groups (4-7, 8-11, 12-15 and 16-20 weeks of age) were eluted using FTA cards and sent to the DIAGNOS® Laboratory (HIPRA). Real-Time Polymerase Chain Reaction (rt-PCR) tests[3,4] were performed for both Bb and Pm detection. The results are presented in 4 different groups based on the bacterial concentration detected by the PCR; none detected (negative), low (+), medium (++) and high (+++). An average of 30 fattening pigs per farm was randomly selected in the slaughterhouse, and the nasal turbinates were evaluated based on the European Pharmacopoeia guidelines.[2] The Wilcoxon test was used for the statistical analysis.

Results

Bb was detected on all the farms (11), whilst Pm was only present on 3 out of 11 (27%) (Table 1).

Table 1. Detection of Bb and toxigenic Pm in pigs from 11 farms.



* Bacterial DNA was detected in low (+), medium (++) and high (+++) amounts.

The farms that were Pm-positive were the ones with highest nasal lesion scores (P-value = 0.04) as shown in the following illustrations (Figure 1).

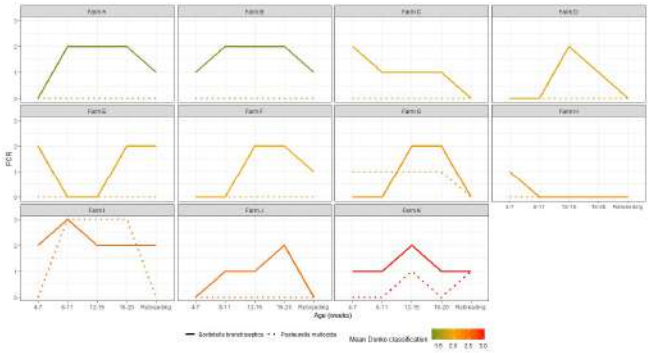


Figure 1. Correlation between the detection of Bb and Pm in oral fluids and the grade of nasal lesion evaluated by the Donko technique.

The mean nasal lesion score was 2.6 whilst the highest grade of lesion (3) was present in 70% of the animals. In the case of Bb-positive farms which were Pm negative, the mean lesion score was lower compared to the Pm-positive farms (1.88) and grade 3 was seen in just 25% of the nasal slices evaluated.

Conclusions and Discussion

The results obtained in this trial show that Bb and Pm are present on Colombian farms irrespective of the location or type of farm. The highest grade of nasal turbinate lesions was detected on farms showing positivity to Pm in oral fluids, so the presence of Progressive Atrophic Rhinitis in the country is a reality. Further studies are needed in order to assess the economic losses caused by atrophic rhinitis and how vaccination against AR could help to avoid these losses.

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Effect of cross-fostering on cell mediated maternal immunity in two-day old piglets

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Introduction

Piglets can be infected with *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) early in life as dam-to-piglet transmission plays an important role (1). Therefore, maternally derived immunity (MDI) received via colostrum may be important to protect against *M. hyopneumoniae* infections in suckling piglets. MDI consists of a humoral and a cell mediated component. Previous studies showed that piglets who are cross-fostered before colostrum intake, receive humoral MDI against *M. hyopneumoniae* but based on a delayed-type hypersensitivity reaction, transfer of cell mediated MDI could not be demonstrated (2). The objective of this study was to investigate the possible transfer of T cell subpopulations in piglets that were either cross-fostered or not.

Materials and Methods

Forty-seven piglets from six commercial hybrid sows of different parities that had been vaccinated against *M. hyopneumoniae* during gestation were included (n=8 pigs per sow). Half of the piglets were moved to another sow immediately after birth (0-6h), before colostrum intake, allowing them to ingest colostrum from another dam.

Blood was collected from the piglets two days (48 hours) after birth. Peripheral blood mononuclear cells (PBMCs) were isolated using a lymphoprep density gradient (Stemcell technologies, Vancouver, Canada) and plated in 24-well plates at 5 x 10^6 cells/well in 1 mL of AIM-V medium (Gibco™, ThermoFisher Scientific, Waltham, MA, USA). The cells were stimulated *in vitro* overnight (18h) with 6.25 x 10^7 CCU/mL of *M. hyopneumoniae* J strain bacterin. Brefeldin A (eBioscience, San Diego, CA, USA) was added for the last 4h of stimulation. Next, the cells were harvested and a staining protocol was performed. First, cells were incubated with a LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit (Invitrogen™, ThermoFisher Scientific, Waltham, MA, USA). Afterwards they were incubated with anti-CD3 DL755, anti-CD4 and anti-CD8 monoclonal antibodies and subsequently with the corresponding secondary antibodies: anti-mouse IgG2b FITC (Biolegend, San Diego, CA, USA) and anti-mouse IgG2a PE-Cy7 (Abcam, Cambridge, UK). Data were acquired with a CytoFLEX flow cytometer (Beckman Coulter, Bea, CA, USA).

Results and Discussion

For the four T cell subpopulations i.e. $CD8^+$, $CD4^+$, double positive cells (DP) and double negative cells (DN), no statistical significant difference between the non-cross-fostered and cross-fostered piglets was observed (Figure 1). The percentage of T cell subpopulations in this study is similar to previous research that isolated PBMCs from one-day old piglets (3).

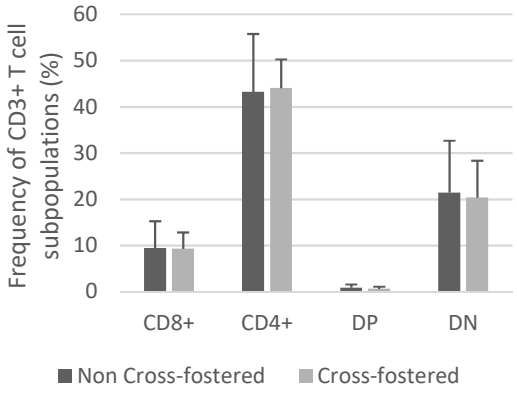

Figure 1. T cell subpopulations isolated from blood of two-day old piglets (n=47) that suckled colostrum from their own mother (n=23) or from another sow (n=24).

The T cell subpopulations are expressed as a percentage out of the total number (100%) of $CD3^+$ cells. DP: double positive DN: double negative.

These results show that two-day old piglets that were moved to another dam before colostrum intake had similar T cell subpopulations in their blood as compared to piglets suckling colostrum from their own mother. The PBMCs were isolated and stimulated *in vitro* with a *M. hyopneumoniae* J-strain. This might explain the difference with studies using the delayed-type hypersensitivity reaction which is an *in vivo* test that might be less sensitive as the currently used *in vitro* test (2).

Conclusions

Cross-fostering of piglets, before colostrum intake, does not result in a lower percentage of the T cell subpopulations in blood of two-day old piglets compared to non-cross-fostered piglets, provided that the new mother is still producing colostrum.

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Cross-sectional study on the prevalence of *Mycoplasma hyopneumoniae* in breeding animals

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Introduction

Mycoplasma hyopneumoniae (*M. hyopneumoniae*) is the primary agent of enzootic pneumonia, a chronic respiratory disease in pigs that causes significant economic losses (1). Infection occurs often early in life as dam-to-piglet transmission plays an important role (2). Previous studies investigated the seroprevalence of *M. hyopneumoniae* in breeding animals and the presence of the pathogen in replacement gilts (3). The objective of this study was to investigate the prevalence of *M. hyopneumoniae* in gilts and sows in different stages of the reproductive cycle.

Materials and Methods

On three commercial Belgian single-site farrow-to-finish herds a cross-sectional sampling was performed in the breeding animals. All animals were group housed from four weeks of gestation onwards till one week before farrowing, gilts were vaccinated against *M. hyopneumoniae* prior to or in the quarantine unit and sows were not vaccinated.

Tracheobronchial swabs (TBS) were taken from 10 gilts and 10 sows of different parities at four different time points of the reproductive cycle i.e. 30-40 and 80-90 days of gestation, 3-5 days after farrowing and at weaning.

After collecting the sample, TBS were stored in a 15 ml falcon with 1 ml of physiologic solution for transport. Afterwards the 1 ml was put in aliquots after vortexing and stored at -80° C till further analysis. DNA was extracted from the TBS using a commercial kit (DNeasy® Blood and Tissue Kit, Qiagen, Venlo, The Netherlands) and a *M. hyopneumoniae* specific nested PCR was performed (4).

A generalized (binary logistic) linear model was used to test the associations of *M. hyopneumoniae* prevalence taking into account the following parameters: farm, time points of the reproductive cycle and parity status (sow or gilt). IBM SPSS statistics 26.0 was used.

Results and Discussion

The results of *M. hyopneumoniae* positive animals are given in table 1. On farm A only four sows and two gilts were sampled after farrowing because the farrowing crate did not allow a safe sample collection.

The prevalence of *M. hyopneumoniae* was 1.52 %, 43.75 % and 3.75 % on farm A, B and C, respectively. On farm B, the prevalence was higher than on the other farms, and positive animals were found at all time-points.

Table 1. Number of nPCR positive TBS samples on total number sampled from breeding animals of three farms.

	Farm A (n=66)		Farm B (n=80)		Farm C (n=80)	
	sow	gilt	sow	gilt	sow	gilt
30-40d gestation	1	0	6	8	0	0
80-90d gestation	0	0	3	7	1	1
3-5d after farrowing	0*	0*	3	2	1	0
Weaning	0	0	3	3	0	0
Total	1	0	15	20	2	1

* Farm A: four sows and two gilts were sampled at 3-5d after farrowing instead of ten.

Both the factors farm and time-point in the reproductive cycle were statistically significant in the model, whereas parity status was not. The prevalence in farm A and C was very low, whereas almost half of the samples in farm B tested positive. This implies that mainly the results of farm B determined the effect of sampling time and parity group. The prevalence at 30-40 days of gestation was significantly ($p=0.05$) higher than at farrowing (10% difference (0-21%, 95% CI)) or at weaning (10% difference (0-21%, 95% C.I.)).

Conclusions

There was a statistically significant difference in *M. hyopneumoniae* prevalence in breeding animals between the farms and between different stages of the reproductive cycle. As this is an ongoing study results of more farms will follow, allowing to provide better insights on a regional (Flanders) level.

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Effect of cross-fostering on cell mediated maternal immunity in two-day old piglets

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Blood was collected from the piglets two days (48 hours) after birth. Peripheral blood mononuclear cells (PBMCs) were isolated using a lymphoprep density gradient (Stemcell technologies, Vancouver, Canada) and plated in 24-well plates at 5 x 10^6 cells/well in 1 mL of AIM-V medium (Gibco™, ThermoFisher Scientific, Waltham, MA, USA). The cells were stimulated *in vitro* overnight (18h) with 6.25 x 10^7 CCU/mL of *M. hyopneumoniae* J strain bacterin. Brefeldin A (eBioscience, San Diego, CA, USA) was added for the last 4h of stimulation. Next, the cells were harvested and a staining protocol was performed. First, cells were incubated with a LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit (Invitrogen™, ThermoFisher Scientific, Waltham, MA, USA). Afterwards they were incubated with anti-CD3 DL755, anti-CD4 and anti-CD8 monoclonal antibodies and subsequently with the corresponding secondary antibodies: anti-mouse IgG2b FITC (Biolegend, San Diego, CA, USA) and anti-mouse IgG2a PE-Cy7 (Abcam, Cambridge, UK). Data were acquired with a CytoFLEX flow cytometer (Beckman Coulter, Bea, CA, USA).

Results and Discussion

For the four T cell subpopulations i.e. $CD8^+$, $CD4^+$, double positive cells (DP) and double negative cells (DN), no statistical significant difference between the non-cross-fostered and cross-fostered piglets was observed (Figure 1). The percentage of T cell subpopulations in this study is similar to previous research that isolated PBMCs from one-day old piglets (3).

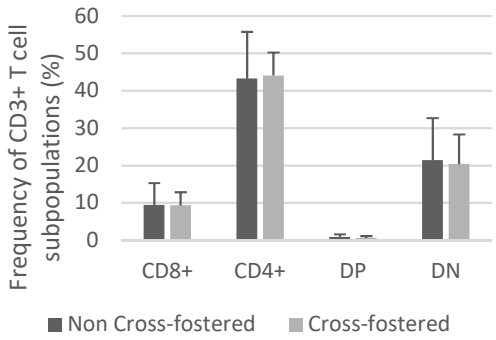

Figure 1. T cell subpopulations isolated from blood of two-day old piglets (n=47) that suckled colostrum from their own mother (n=23) or from another sow (n=24).

The T cell subpopulations are expressed as a percentage out of the total number (100%) of $CD3^+$ cells. DP: double positive DN: double negative.
These results show that two-day old piglets that were moved to another dam before colostrum intake had similar T cell subpopulations in their blood as compared to piglets suckling colostrum from their own mother. The PBMCs were isolated and stimulated *in vitro* with a *M. hyopneumoniae* J-strain. This might explain the difference with studies using the delayed-type hypersensitivity reaction which is an *in vivo* test that might be less sensitive as the currently used *in vitro* test (2).

Conclusions

Cross-fostering of piglets, before colostrum intake, does not result in a lower percentage of the T cell subpopulations in blood of two-day old piglets compared to non-cross-fostered piglets, provided that the new mother is still producing colostrum.

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Use of qPCR to evaluate the presence of type B *Clostridium novyi* in sudden death sows and healthy sows

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Introduction

Type B *Clostridium novyi* causes porcine infectious necrotic hepatitis, inducing sudden death (SD) in breeding sows. Little is known about the epidemiology of the disease, and its diagnosis is controversial since *C. novyi* is considered to be a common and early postmortem invader[1]. Because isolation of this bacterium is not always successful due to its strict anaerobic requirements, and autolysis is inherent to the necrotic liver, the identification of *C. novyi* by PCR is a valuable tool to provide an aetiologic diagnosis. The aim of this study was to adapt a Type B *C. novyi* real time PCR (qPCR), as a tool to detect C*. novyi* in sows with and without suspected infection.

Materials and Methods

A previously described PCR[2] assay was adapted to the SYBR® Green-based qPCR methodology. Validation was performed by testing the following specimens:

A. **Bacterial strains:** A panel of 40 reference strains, including 31 non-type B *C. novyi* and *C. haemolyticum*, as well as 9 type B *C. novyi*. A set of 10-fold serial dilutions of the Type B *C. novyi* ATCC25758 strain was also tested.
B. **Samples from sudden death sows:** Fifty liver samples from breeder sows on 25 unrelated commercial farms in Spain, France and Belgium. They were collected either fresh (29) or desiccated on FTA cards (21), from sows with suspected *C. novyi* infection based on gross lesions after SD.
C. **Samples from healthy sows:** Individual rectal swabs from 270 healthy breeder sows housed on nine unrelated commercial farms in Spain, and 88 fresh liver samples from healthy breeding sows collected at slaughter (diverse origins).

Samples were subjected to automatic DNA extraction/purification (QIAamp DNA minikit in the Qiacube 2.0. Qiagen) and qPCR amplification (SYBR® Green qPCR Kit. Qiagen). The threshold cycle (Ct) value was determined for each sample.

Results

The qPCR was 100% specific to type B *C.novyi*, with a limit of detection of 10^3 CFU/mL and efficiency of 95.6%.

Table 1. Results of the qPCR assay on samples of diverse nature and condition.

Origin	Sample type	Positive/ Total (%)
Bacterial culture	Type B *C. novyi* single CFU	9/9 (100)
Bacterial culture	Non-type B *C. novyi* single CFU	0/31 (0)
Sudden death sows	Fresh liver	19/29 (65.5)
	Desiccated liver (FTA card)	19/21(90.5)
Healthy sows	Fresh liver	0/88 (0)
	Rectal swab	0/270 (0)

CFU = colony forming unit

Conclusions and Discussion

The qPCR assay was successfully adapted and validated, demonstrating suitability for the specific detection of Type B *C. novyi* DNA in both fresh and desiccated specimens. Additionally, results from this study demonstrate the usefulness of the FTA cards for room temperature transportation of complex biological samples such as necrotic liver in field cases of *C. novyi*-associated SD.

None of the samples, supposedly targets for *C. novyi,* such as the liver and rectal swabs from healthy sows, were found to be infected by this qPCR. These results differ from those reported in previously published studies dealing with this pathogen in pigs. Specifically, the assumption that *C. novyi* is a normal inhabitant of the posterior gut and liver of healthy pigs[3], is not supported in this study, suggesting that type B *C. novyi* infection is not ubiquitous.

All in all, this study demonstrates that qPCR would considerably improve the diagnosis of *C. novyi*-associated SD in sows, and provides relevant information for the establishment of preventive measures such as vaccination.

Acknowledgements

The authors wish to thank the farmers for their support, and the UCAM-Diagnos staff for sample processing.

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Coinfections between virulence factors of *Escherichia coli* and *Clostridium perfringens* type C

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Introduction

Enterotoxigenic *E.coli* (ETEC) remains one of the most important primary pathotypes causing neonatal diarrhea in piglets from 0 to 4 days of age (1). For this, ETEC produces several virulence factors such as fimbrial adhesins and enterotoxins, which are responsible for the adhesion of the bacteria in the endothelium and the subsequent release of toxins in the intestinal lumen (2). Through vaccination, colostrum immunoglobulins are intended to block the adhesin function so that bacterial attachment to the intestinal epithelium and, consequently, intestinal colonization, are prevented. Additionally, milk antibodies against the toxins also neutralize their toxic effect in the gut of the piglet (3). On the other hand, *Clostridium perfringens* type C is a primary pathogen that produces CPA and CPB toxins, CPB being the main virulence factor (4). The aim of this study was to evaluate the prevalence of ETEC and *Clostridium perfringens* type C (CpC) as well as the number of coinfections between the virulence factors in piglets suffering from neonatal diarrhea.

Materials and methods

A total of 1010 fecal samples, (3 samples per farm on average), from 39 different countries [America (n=358), Asia (n=155), Europe (n=478) and South Africa (n=19)] were analyzed and included in this study. The samples had been obtained between January 2014 and December 2019, and were subsequently sent to the DIAGNOS® Laboratory using FTA® ELUTE cards (Whatman Inc., Florham Park, NJ). A multiplex polymerase chain reaction (PCR) test, adapted from previous studies, was performed to detect genes encoding F4, F5, F6 adhesion factors, the heat-labile toxin (LT) of ETEC and β-toxin of CpC (5,6). Finally, once all the results had been obtained, an exploratory analysis was performed in order to describe the prevalence and coexistence of the different antigenicity factors.

Results

From all the samples analyzed, 693 (68.61%) were positive for some of the antigenicity factors. From those 693 positive samples, the LT toxin and the F4 fimbriae showed the highest prevalence (39.1% and 10.5% respectively) (Table 1). Therefore, the strongest correlation between virulence factors was found between the LT toxin and fimbriae factors that coinfected neonatal piglets in 306 (44.2%) of the samples (Figure 1).

Table 1. PCR test summary, results obtained from 2014 to 2019 in HIPRA DIAGNOS®.

	ETEC				CpC
	F4	F5	F6	LT	B toxin
Positive result	536	92	81	399	43
Negative result	478	922	933	615	971

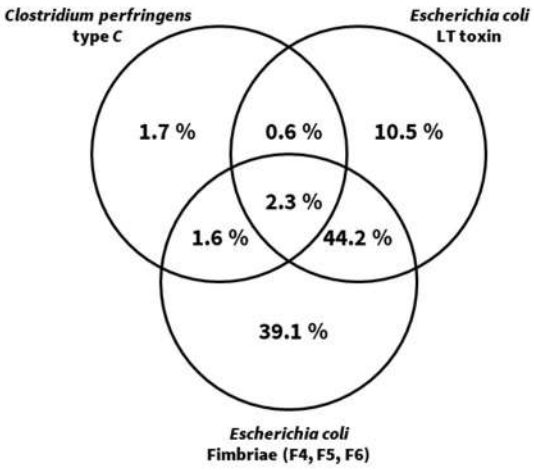

Figure 1. Venn Diagram showing the coexistence of virulence factors from positive samples (n = 693)

Conclusions and discussion

F4 and heat-labile toxin (LT) were detected in most of the samples analyzed; both virulence factors were detected in 44.2% of all positive samples. Immunization against all these virulence factors is imperative for optimal protection of piglets during their first days of life.

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Evaluation of the Porcine Gut Microbiome Response to *Lawsonia intracellularis* Infection

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Introduction

Lawsonia intracellularis is a Gram-negative intracellular bacterial pathogen and the causative agent of porcine proliferative enteropathy (PPE). Infection with *L. intracellularis* has been estimated to occur in more than 90% of swine farms worldwide.[1] PPE can lead to diarrhea, intestinal lesions and significant production loss.[1] Previous research has suggested that gut microbiome composition is an important factor in the establishment of *L. intracellularis* infection.[2] The gut microbiome is known to influence health as well as production performance of swine.[3] It is also known that the composition of the microbiome varies between different portions of the intestinal tract and the ileum is the preferential site of *L. intracellularis* infection.[1,3,4] To better understand the relationship of *L. intracellularis* infection and the microbial community of the gut, we evaluated the microbiome response of the small and large intestine of pigs experimentally challenged with *L. intracellularis* at different stages of infection.

Materials and Methods

Two treatment groups were evaluated: one treatment group received *L. intracellularis* challenge and the other treatment group served as a non-infected control. Animals that received challenge were inoculated with a gut homogenate containing approximately 4 x10^7 *L. intracellularis* organisms at six weeks of age. Fecal samples were collected at time of challenge and sequential necropsy was performed on 6 pigs per treatment group at 7, 21 and 28 days post infection (dpi) to evaluate microbiome response at different stages of infection. At each necropsy, samples collected for microbiome analysis were: digesta of the terminal ileum, mucosal scraping of the terminal ileum, cecal digesta and feces. After DNA extraction, microbiome analysis was performed by analyzing the V4 region of the 16S rRNA gene using DADA2 and phyloseq packages in R.[5] *L. intracellularis* infection was confirmed by performing immunohistochemistry (IHC) of intestinal tissue, measurement of fecal shedding and seroconversion.

Results and Discussion

PPE was successfully reproduced as measured by characteristic lesions, detection of *L. intracellularis* by IHC, shedding by PCR and seroconversion in the challenged group. The negative control group remained negative for all diagnostic measures of *L. intracellularis* throughout the trial.

Beta diversity results indicate that *L. intracellularis* infection led to a significant change in the microbial community composition of both the small and large intestine as measured by the Bray Curtis Dissimilarity Metric, which was visualized using principle coordinate analysis (PCoA) (community composition) differences (PERMANOVA $p<0.05$). In ileum mucosal samples, differences between treatments were more evident and significant at later time points (21dpi, $p=0.006$; 28dpi, $p=0.003$) when measures of *L. intracellularis* infection were increased as compared to the earlier time point of infection (7dpi, $p=0.4$) (Figure 1). Significant differentially abundant bacteria were also found between infected and non-infected groups, suggesting they could play a role in disease.

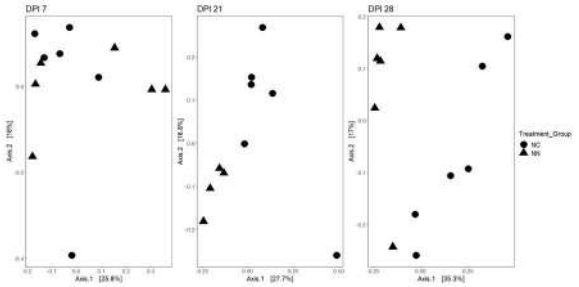

Figure 1. PCoA of Bray Curtis Dissimilarity Metric of ileum mucosal samples at 7, 21 and 28 days post *L. intracellularis* infection. DPI=days post infection; NC= challenged with *L. intracellularis*; NN= Not challenged

Conclusions

Evaluating differences in microbial composition further will lead to a better understanding of PPE and the interactions of *L. intracellularis* with other bacteria present during infection and their potential interaction in promoting disease and its negative consequences on health and production performance.

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Investigating the Shedding, Transmission and Seroconversion to an Oral Live Vaccine Against *Lawsonia intracellularis*

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Introduction

Porcine proliferative enteropathy (PPE) or Ileitis is a disease caused by *Lawsonia intracellularis*. PPE is a significant enteric disease of swine with estimated prevalence of over 90% worldwide[1]. Disease can lead to significant loss in production performance as well as diarrhea and mortality[1]. Aid in prevention of disease can be accomplished by the use of a commercial orally delivered live attenuated vaccine Enterisol® Ileitis (Boehringer Ingelheim Vetmedica, Inc.)[1,2]. Previous research has found little to no shedding of the vaccine strain in swine[2,3]. However, the development of more sensitive diagnostic tests warrants investigation to update expectations to aid in interpreting diagnostic results from vaccinated animals. Along with importance from a diagnostic perspective, understanding transmission of the vaccine is also important for research trial design to minimize vaccine exposure between treatment groups. The objective of this study was to investigate if the vaccine strain of Enterisol® Ileitis can be detected by PCR in feces, if transmission can occur to non-vaccinated animals and if seroconversion occurs due to vaccination.

Materials and Methods

Forty-one pigs were randomly assigned to either vaccinated (n=21) or non-vaccinated treatment groups (n=20). All animals were housed in one pen to allow comingling between treatment groups and maximize chances of detecting transmission. At three weeks of age, vaccinated animals were administered a 2 ml oral dose of Enterisol® Ileitis by drench, the other half of animals in the pen were left unvaccinated. Fecal samples were collected from all animals at 0, 1, 3, 7, 14, 21, 28, 35, and 49 days post-vaccination (dpv) for quantitative *Lawsonia* PCR. Serum sampling occurred at day 0, 35 and 49 dpv to investigate seroconversion by ELISA.

Results

Investigating fecal shedding over time, we observed among vaccinated animals that most fecal shedding occurred at 1 dpv with 76% (16/21) of animals with positive fecal samples with values ranging from < 1,400 to $7.92x10^3$ organisms per gram of feces (Figure 1). The 1 dpv time point was also when greatest number of non-vaccinated animals was found to shed *Lawsonia*, with 15% (3/20) of fecal samples being positive with values at <1,400 organisms per gram of feces. Following 1 dpv, we observed a decline in fecal shedding in vaccinated animals with only 5 animals sporadically shedding *Lawsonia* between 28 and 49 dpv with levels ranging from < 1,400 to $1.04x10^5$. The non-vaccinated group only had one animal with a positive fecal sample after 1 dpv, detected at 49 dpv with a value of $9.15x10^3$. No seroconversion was observed in any animals.

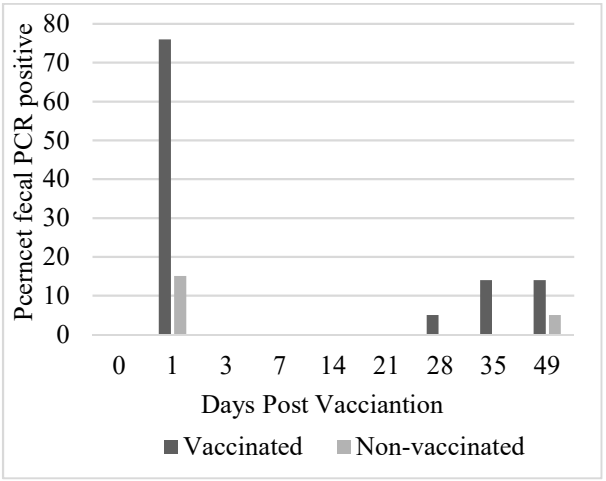

Fig ure 1. Fecal shedding of *Lawsonia intracellularis* detected over time by PCR.

Conclusions and Discussion

Fecal shedding of *Lawsonia* was detected in vaccinated animals mostly on the day following vaccination, only a few animals had detectable shedding after that time point until 7 weeks post vaccination (Figure 1). Minimal shedding was detected in non-vaccinated animals suggesting that there was minimal transmission of vaccine strain from vaccinated to non-vaccinated animals in close contact. Although all animals were fecal PCR negative prior to vaccination, the possibility of exposure to a field strain of *Lawsonia*, especially after 1 dpv cannot be ruled out. This data suggests that while shedding of *Lawsonia* can occur in vaccinated animals, in the time points evaluated and conditions of this study, there was minimal to no transmission of Enterisol® Ileitis from vaccinated to non-vaccinated pigs and vaccination did not lead to seroconversion.

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Comparative Field Evaluation of Bayovac® MH-PRIT-5 ONE in nursery pigs

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Introduction

Enzootic pneumonia is a major swine respiratory disease with worldwide distribution. The causative agent is *Mycoplasma hyopneumniae* and the most frequent clinical sign is dry, non-productive coughing. The disease causes growth retardation, increased feed conversion, batch unevenness (1) and increases susceptibility to other respiratory infectious agents, leading to the development of the Porcine Respiratory Disease Complex (2). Control of *M. hyopneumoniae* pneumonia can be achieved by improvement of farm management (3), strategic antibiotic treatment and vaccination. Proper vaccination can reduce clinical symptoms, antibiotic usage and the severity of lung lesion, improving well-being and performance (4). Bayovac® MH-PRIT-5 ONE (Bayer Animal Health) is a new single dose vaccine, containing 10^{10}ccu of inactivated bacteria in an oil adjuvant. In this study, the efficacy of Bayovac® MH-PRIT-5 ONE in the nursery period was compared, under field conditions, to an already available commercial vaccine (inactivated bacteria combined with oil adjuvant).

Materials and Methods

Piglet batches were assigned to two groups. Piglets in the Bayovac® MH-PRIT-5 ONE group were vaccinated (i.m) at 3 weeks of age, following label recommendations. Piglets in the Vaccine B group were vaccinated (i.m) at 7 days of age, according to the product's label. Serum samples were randomly collected at 3, 12 and 27 weeks of age. Anti-*Mycoplasma* antibody titers were analyzed by ELISA (IDEXX). Body weight was recorded at the day of entry and exit of the nursery room. Average daily weight gain (ADWG) was calculated for the entire nursery period. The culling rate was recoded from weaning until end of the nursery.

Results

In total, two batches of 472 and 466 piglets each were included in the Bayovac® MH-PRIT-5 ONE group. These batches were weaned at 23 and 26 days of age, weighing on average 8.09 and 7.64kg respectively (7.87kg group average). A total of 766 Bayovac® piglets reached the end of nursery at 76 days of age with average of 26.66kg, resulting in a 18.34% culling rate. Culling rate was higher than normally observed in the farm due to a diagnosed PRRS outbreak. The ADWG during the nursery period was 364.85 g/day. For the Vaccine B group, two batches of 468 and 466 piglets each were included. These batches were both weaned at 38 days of age, weighing on average 8.46 and 8.06kg respectively (8.26kg group average). A total of 744 piglets reached the end of nursery at 84 days of age with average of 23.35kg, resulting in a 20.34% culling rate, which was also influence by the ongoing PRRS outbreak. The ADWG during the nursery period was 324.52 g/day.

Table 1. Performance of nursery pigs in each group.

Group	ADWG (g/day)*	Culling (%)
Bayovac® MH-PRIT-5 ONE	364.85	18.34
Vaccine B	324.52	20.34

* p=0.057

M. hyopneumoniae antibodies were not detected at 3 weeks of age in both groups (Figure 1). Antibodies slightly increase at 12 weeks, with complete seroconversion of the batches at 27 weeks of age.

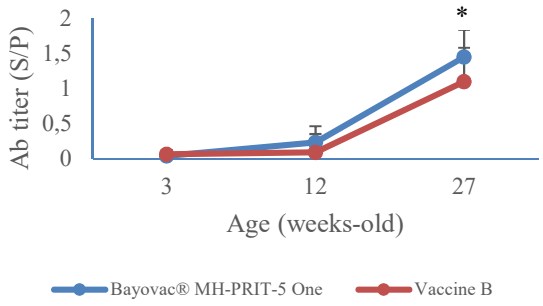

Figure 1. Profile of *M. hyopneumoniae* antibodies (* p<0.05).

Conclusions and Discussion

Vaccination against *M. hyopneumoniae* remains one of the most important aspects in controlling the incidence of swine respiratory disease. Despite the concomitant PRRS outbreak, the current field evaluation showed that Bayovac® MH-PRIT-5 ONE vaccine can provide adequate protection to piglets, ensuring a good growth performance and lower culling in the nursery period.

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Seroprevalence and risk factors associated with infection by *Lawsonia intracellularis* in backyard pigs in the state of Minas Gerais, Brazil in 2016

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Introduction

Lawsonia intracellularis is the causative agent of proliferative enteropathy, an endemic disease responsible for economic losses in swine industrial herds(1) worldwide. This enteropathogen causes diarrhea in pigs and several other animal species. The presence and circulation of this microorganism in backyard production systems is unknown and it may be an important factor in the maintenance of this enteropathogen. The aim of this study was to evaluate seroprevalence and risk factors associated with *L. intracellularis* infection in backyard pigs in the state of Minas Gerais, Brazil.

Materials and Methods

One thousand and one hundred serum samples were collected from backyard pigs ranging from 2 to 6 months of age, originated from 288 farms distributed among 12 mesoregions of the state of Minas Gerais, Brazil. Sampling occurred from September to December 2016. These animals did not have any evident signs of diarrhea. Ten risk factors were evaluated in each herd: proximity to natural reserves; areas of environmental protection or national parks with wild swine fauna; peri-urban areas or poor communities; areas with swine raised extensively; rural settlements or indigenous reserves; supply of food waste (washing) to swine; proximity to dumps; owner with property in another country; proximity to grease fittings; proximity to quarantine of swine. Serum samples were tested for the presence of anti-*Lawsonia intracellularis* antibodies (IgG) using the immunoperoxidase monolayer assay (IPMA)(2). Serum titration were performed in strong positive samples. A Poisson Regression model was conducted to evaluate the risk factors through multivariate analysis.

Results

The overall weighted seroprevalence was 97.7% (CI 95%: 96.7 – 98.46). Seroprevalence by mesoregions of the state of Minas Gerais is shown in Table 1. Due to the high prevalence, only samples used for titration (131 samples) were chosen to carry out risk factors analysis. Most IgG titers were 1:120 (60/132) and 1:480 (62/132), with the lowest antibody titer being 1:30 and the highest 1:7680. Among the 10 criteria for risk, only the interaction of the "peri-urban areas or poor communities", with "areas with swine raised extensively" and the variable "proximity to city dumps" were statistically relevant ($p < 0.05$) (Table 2).

Table 1. Seroprevalence of *Lawsonia intracellularis* infection in different mesoregions of the state of Minas Gerais, Brazil.

MESORREGION	SAMPLED CREATORIES	SAMPLED ANIMALS	SEROPREVALENCE	CI 95%
Campo das Vertentes	11	33	90.91	75.6 – 98.0
Central Mineira	10	40	92.11	78.6 – 98.3
Vale do Mucuri	12	52	93.70	82.8 – 98.6
Sul/Suldoeste	27	68	95.71	87.9 – 99.1
Zona da Mata	28	63	96.80	87.9 – 99.6
Metropolitana	29	112	97.25	92.1 – 99.4
Jequitinhonha	25	82	97.47	91.1 – 99.6
Oeste de Minas	14	50	98.00	89.3 – 99.9
Norte de Minas	18	68	98.33	91.0 – 99.9
Noroeste de Minas	29	95	98.90	94.0 – 99.9
Triângulo Mineiro	70	409	99.25	98.0 – 99.8
Vale do Rio Doce	10	28	100.0	–
Total: 12	288	1100	97.77	96.7 – 98.46

Table 2. Risk factors for high *anti-Lawsonia intracellularis* titers in pigs from farms in Minas Gerais.

MODEL	RISK FACTORS	P value	REGRESSION COEFFICIENT	STANDARD ERROR	CI
0 - 0	****Outside** the periurban area or communities and **outside the area** with swine raised extensively	Base line	-	-	Baseline
0 - 1	****Outside** the periurban area or communities and **within** areas with swine raised extensively	0,513	1,2	0,34	0,61 – 2,11
1 - 0	****Within** periurban areas or communities and **outside the area** with swine raised extensively	0,021	1,8	0,46	1,09 – 2,98
1 - 1	****Within** periurban areas or communities and **within** areas with swine raised extensively	0,000	3,7	0,86	2,40 – 5,89
	Proximity to dumps	0,052*	2,2	0,89	0,99 – 4,89

* maintained in the model, because it is an important variable.
** interaction of these two variables.

Conclusions and Discussion

Based on the results, there is a high circulation of the agent in backyard swine production systems farms in the state of Minas Gerais, Brazil. Our findings show that the interaction of animals raised near peri-urban areas, raised extensively and in proximity to landfills, are risk factors for the spread of the agent.

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Prevalence of the pathogenicity factors of the Enterotoxigenic *Escherichia coli* and *Clostridium perfringens* type C per PCR from 2017 to 2019 in Brazil

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Introduction

Escherichia coli and *Clostridium perfringens* are part of the intestinal microbiota of swine, a dysbiosis-causing diarrhea related to these agents (1). For a correct diagnosis, it is important to check the presence of pathogenicity factors as the bacterial isolation only does not indicate that these agents are causing diarrhoea as they are commensals of the bacterial flora. One of the most effective techniques used is the detection of virulence factors, such as the presence of fimbriae F4, F5, F6, the heat-labile toxin (LT) of *E. coli* and the toxin *β* of *C. perfringens* type C, through a quantitative PCR. The purpose of this study was to investigate the prevalence of pathogenicity factors of Enterotoxigenic *E. coli* (ETEC) and *C. perfringens* type C, as the main case of young piglet diarrhea through fecal samples collected between 2017 and 2019.

Materials and methods

From January 2017 to October 2019, 245 fecal samples were collected from suckling pigs with symptoms diarrhea, from different regions of Brazil. The samples were impregnated with FTA ELUTE cards (Whatman Inc., Florham Park, NJ), sent to DIAGNOS (Laboratório Hipra Saúde Animal) and processed with the multiplex Polymerase Chain Reaction (PCR) technique to detect the genes that codify the adhesion factors F4, F5, F6 and the LT toxin of *E. coli* and *β* toxin of *C. perfingens* type C.

Results

Among the 245 samples analyzed, 78 (31.84%) were positive and 167 (68.16%) were negative for the pathogenicity factors (Figure 1). The prevalence of these pathogenicity factors in the 78 positive samples was 35.90% for F4; 2.56% for F5; 19.23% for F6; 29.49% for LT and 12.82% for *β* toxin (C. *perfringens*), as illustrated in Figure 2. The values found are similar to the reports of prior works (3). In newborn piglets, the strains of ETEC usually present the fimbriae F4, F5 and F6. The F4 receptors are expressed in swine's enterocytes during their whole life. The receptors for fimbriae F5 and F6 are expressed with lesser intensity in older piglets (2). As regards to LT toxin, the genes involved in their synthesis are located in the plasmids where the genes for F4 (4) are also found. The *E.coli* adhere to the intestinal cells via specific receptors, then, the LT enterotoxin is produced in the intestinal mucous membrane and links to the receptors in the surface of the enterocyte, activating the adenyl cyclase, causing the secretion of sodium chloride and water by osmosis, generating a condition of secretory diarrhea (2). The passive immunity against enteric infections comes from the colostrum and milk; the antibodies inhibit the bacterial adherence to the receptors in the enterocytes and neutralize the activities of the enterotoxins produced by *E. coli* and *C. perfringens*. A correct sow immunization, correct piglet colostrum and milk intake and the infection pressure control could reduce the incidence of infectious diarrhea in the piglets.

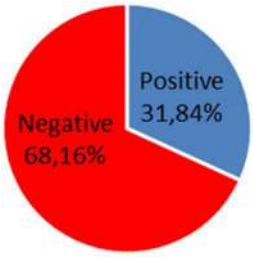

Figure 1: Results of PCR test conducted in the DIAGNOS from 2017 to 2019.

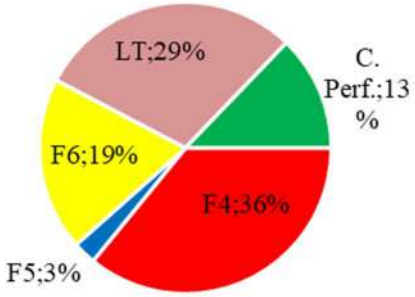

Figure 2: Distribution of PCR test positive per pathogenicity factors

Conclusions and discussion

The multiplex PCR is a crucial tool to aid the diagnosis of new-born diarrhea, the positivity for the pathogenicity factors of *E. coli* and/or of *C. perfringens*, can indicate the involvement of these agents in the conditions of diarrhea.

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Serological survey of the humoral immune response to *Erysipelothrix rhusiopathiae* on commercial farms in Brazil

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Introduction

The protective role of specific antibodies against Swine Erysipelas (SE) enhanced through vaccination is the key to controlling infectious reproductive problems in sows[1]. Therefore, the presence of breeding animals seronegative to SE on vaccinated farms causes concern among farmers, since these negative animals are susceptible to suffering clinical signs related to SE[2], which could cause major reproductive losses.

The aim of this study was to compare the humoral immune response against SE elicited by different commercial reproductive vaccines against SE, PPV and/or *Leptospira interrogans sp*., under field conditions in Brazil.

Materials and Methods

The study was split into two parts, the first one evaluating serconversion against SE on farms vaccinating with bivalent reproductive vaccines (against SE and Porcine Parvovirus (PPV)), and the second part, which is the most common scenario, on farms vaccinating with trivalent reproductive vaccines (against SE, PPV and *Leptospira interrogans sp*.). All the samples were from gilts and sows of different parities in the middle of the gestation period. Farms were sampled from 2016 until 2019.

Bivalent vaccine farms: 10 farms that were vaccinating with Vaccine A (ERYSENG® PARVO) with a total of 108 samples and Vaccine B (adjuvanted with α-tocopherol-acetate), with 218 samples, were analyzed.

Trivalent vaccine farms: 101 farms that were vaccinating with Vaccine C (ERYSENG® PARVO/LEPTO) with 2,160 samples, Vaccine D (oil-based adjuvant) with 746 samples, Vaccine E (aluminum hydroxide adjuvant) with 934 samples and Vaccine F (Brazilian vaccine adjuvanted with aluminum hydroxide) with 846 samples, were analyzed.

Results:

With regard to the bivalent vaccines, ERYSENG® PARVO (EP) had higher SE titers (mean of 64.11) compared to vaccine B (mean of 52.66) with statistically significant differences (T-test; *p-value*<0,05). The median of EP remained above the cut-off as seen in Figure 1.

With regard to the trivalent vaccines, ERYSENG® PARVO/LEPTO had higher SE titers (mean of 63.5) compared to Vaccine D (mean of 57.13), Vaccine E (mean of 38.55) and Vaccine F (mean of 48.85) with statistically significant differences (Anova test; *p-value*<0.05).

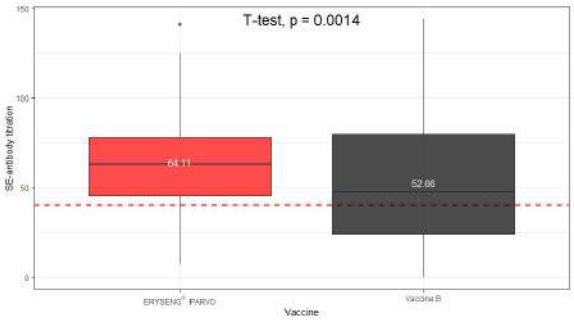

Figure 1. Boxplots of SE serology of farms using bivalent vaccines. Cut-off: 40. Different subscripts show statistically significant differences (T-test; p-value<0.05).

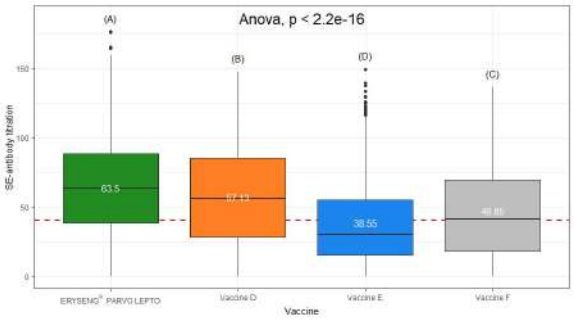

Figure 2. Boxplots of SE serology of farms using trivalent vaccines. Cut-off: 40. Different subscripts show statistically significant differences (Anova test; p-value<0.05).

Conclusions and Discussion

The humoral immune response against *E. rhusiopathiae* elicited by the vaccines tested varied in intensity, the highest being exhibited by ERYSENG® PARVO and ERYSENG® PARVO/LEPTO. This could be due to a different recognition of the antigen by the animals, or a different effect of the adjuvants.

Furthermore, having higher SE titers may suggest lower negative subpopulations[3], meaning a lower risk for the breeding herd.

Acknowledgments

The authors wish to thank HIPRA's HIPRASTATS department for their statistical assistance.

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Detection of *Bordetella bronchiseptica* and *Pasteurella multocida* through oral fluid in growing pigs in the period of 2014 to 2019 in Brazil

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Introduction

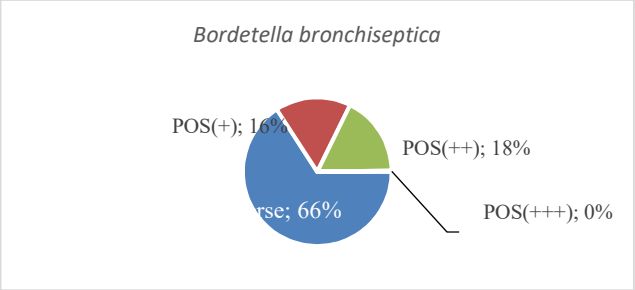
Bordetella bronchiseptica (Bb) is the primary agent of Atrophic Rhinitis (AR), major respiratory disease that attacks growing pigs throughout the world. This bacterium sticks to the nasal mucous membrane and produces a dermonecrotic toxin capable of causing partial loss of bones in the nasal concha, associated with facial distortion and nasal hemorrhage[1]. These lesions predispose the animals to other infections, mainly by *Pasteurella multocida* (Pm), which aggravates the lesions in the nasal concha leading to Progressive Atrophic Rhinitis (PAR), but also by *Haemophilus parasuis, Streptococcus suis* and even *Actinobacillus pleuropneumoniae*[2]. The main form of transmission of the disease is from the sows to their newborn pigs, via nasal contact and further during lactation. The infected piglets could transmit the disease to other pigs in the nursery after weaning[2]. Thus, the losses associated to AR induce a low feeding efficiency and an increase in time for pigs to reach the slaughter weight in relation to healthy animals[3]. The purpose of this study was to evaluate the prevalence of Bb and PM found in samples of oral fluid (OF) collected from 2014 to 2019 in Brazil.

Materials and methods

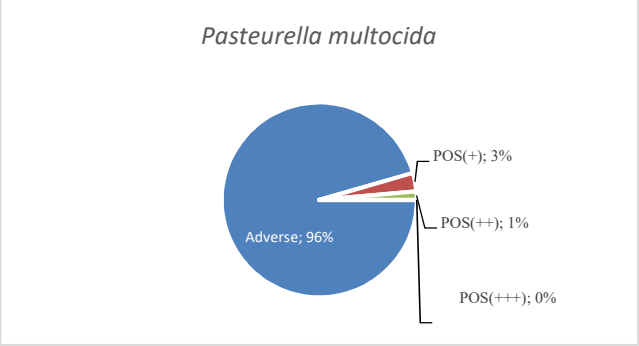
In the period from January 2014 to October 2019, 559 samples of oral fluid were collected from growing pigs from farms in different regions of Brazil. The samples were inoculated in FTA ELUTE cards (Whatman Inc., Florham Park, NJ), sent to DIAGNOS laboratory, (HIPRA Saúde Animal) and analyzed with quantitative-PCR[4,5] the presence of Bb and PM; toxigenic strains of Bb and the dermonecrotic toxin of Pm.

Results

Results are indicated in crosses, the low concentration of DNA is represented by 1 cross (+), concentration mediated in 2 crosses (++) and high concentration in 3 crosses (+++). Among the 559 samples analyzed, 191 (34%) were positive for Bb and 25 (4%) positive for Pm. Distribution of results is represented in graphs 1 and 2, respectively.



Graph 1. Distribution of positiveness for Bb.



Graph 2. Distribution of positiveness for Pm.

Conclusion and discussion

These results indicate the presence of pathogenic strains of Bb in high concentration and low prevalence of Pm in growing pigs in different farms in Brazil. The early detection of these agents can be a favorable strategy in the control of Atrophic Rhinitis before the animals arrive to the slaughterhouse. As other studies suggested, prevention of Atrophic Rhinitis relies on the correct immunization of the sows and the infection pressure control in the environment.

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Serotyping and Antimicrobial Resistance of *Actinobacillus pleuropneumoniae* Isolated from Pigs in Taiwan

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Introduction

Actinobacillus pleuropneumoniae (App) can cause fatal fibrinohemorrhagic and necrotizing pleuropneumonia in swine of all ages and result in huge economic losses. To date, 18 serovars and 2 biotypes of App have been recognized in previously published studies, but the research to identify the serotype of App isolated in Taiwan in recent years was rare. Also, the virulence of App might be related to the serotype; therefore, to analyze serotypes or related genotypes must be taken seriously. Although we have vaccines to prevent actinobacillosis, antimicrobial drugs may be a common treatment to control the disease. To decrease drug resistance, this study was performed to establish treatment guidelines for clinical veterinarians.

Materials and Methods

In this study, a total of 112 strains of App were isolated from diseased swine with typical hemorrhagic necrotizing pneumonia which were sent to the Animal Disease Diagnostic Center of National Chiayi University in Taiwan during 2012 to 2019. Besides, with the development of technology, there were many methods to classify bacterial genes, such as PFGE and AFLP; in this study, we use PCR to identify serotypes. Serotyping was performed by PCR method according to Bossé *et al*. indicated in 2018 [1]. Then, The minimal inhibitory concentration (MIC) test was evaluated by using the broth microdilution which was performed according to Clinical and Laboratory Standards Institute (CLSI) operating rules. The following antimicrobial drugs were selected for antimicrobial susceptibility testing: ampicillin, ceftiofur, cefazoline, doxycycline, enrofloxacin, florfenicol, lincospectin, tilmicosin, tylosin, tiamulin and sulfamethoxazole-trimethoprim.

Results

Among the total of 112 App clinical isolates, 48 strains belonged to serotype 1, followed by serotype 5, serotype 15, serotype 2, and serotype 7. The distribution of serotype according to year was shown in Table 1. And, the results of antimicrobial susceptibility testing of the 85 App strains isolated from pigs from 2012 to 2018 were shown in Table 2.

Conclusions and Discussion

Serotype 1 was the predominant isolate in this study, and we could isolate the serovar 7 and 15 that haven't been isolated in 2011 [2]. According to the results of MIC, we could find App were resistant to many antimicrobial agents Therefore, clinical veterinarians should only select and use antimicrobial agents effective to App to reduce the occurrence of drug resistance in bacteria.

Table 1. Distribution of serotypes according to year.

Serotype	Number of isolates							
	2012	2013	2014	2015	2016	2017	2018	2019
1			8	13	7	12	7	1
2				3		2		
5	1	1	4	1	2	6	16	1
7				1				
15						7	8	11

Table 2. MIC of 11 antimicrobial agents for 85 App strains isolated from pigs from 2012 to 2018.
[a]: No breakpoint data for App is available.

Antimicorbial agents	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Resistance rate (%)
Ampicillin	2	512	52.94
Cefazolin	1	4	-[a]
Ceftiofur	0.25	0.25	1.18
Doxycycline	2	8	61.18
Enrofloxacin	0.25	8	45.88
Florfenicol	8	128	52.94
Lincospectin	16	32	-[a]
Tiamulin	8	16	7.06
Tilmicosin	8	16	7.06
Trimethoxazole-sulfamethoxazole	8	256	77.65
Tylosin	32	64	-[a]

Acknowledgments

Thanks to the the Animal Disease Diagnostic Center of National Chiayi University and all the members of the Bacteriology Laboratory in the department of Veterinary Medicine, National Chiayi University, Taiwan for supporting the study.

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2. Yang CY et al., 2011. J Vet Med Sci 73: 205-208.

Serotypes and Antimicrobial Resistance of *Actinobacillus pleuropneumoniae* Isolated from Pigs in Taiwan

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[1]*Department of Veterinary Medicine, National Chiayi University, Chiayi City, Taiwan.*
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Introduction

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Materials and Methods

In this study, a total of 112 strains of App were isolated from diseased swine with typical hemorrhagic necrotizing pneumonia, which were sent to the Animal Disease Diagnostic Center of National Chiayi University in Taiwan during 2012 to 2019. Besides, with the development of technology, there were many methods to classify bacterial genes, such as PFGE and AFLP; in this study, we use PCR to identify serotypes. Serotyping was performed by PCR method according to Bossé *et al.* indicated in 2018 [1]. Then, The minimal inhibitory concentration (MIC) test was evaluated by using the broth microdilution which was performed according to Clinical and Laboratory Standards Institute (CLSI) operating rules. The following antimicrobial drugs were selected for antimicrobial susceptibility testing: ampicillin, cefazoline, ceftiofur, doxycycline, enrofloxacin, florfenicol, lincospectin, tiamulin, tilmicosin, sulfamethoxazole-trimethoprim and tylosin.

Results

Among the total of 112 App clinical isolates, 48 strains belonged to serotype 1, followed by serotype 5, serotype 15, serotype 2, and serotype 7. The distribution of serotype according to year was shown in Table 1. And, the results of antimicrobial susceptibility testing of the 85 App strains isolated from pigs during 2012 to 2018 were shown in Table 2.

Conclusions and Discussion

Serotype 1 was the predominant isolate in this study, and we could isolate the serovar 7 and 15 that haven't been isolated in 2011 [2]. According to the results of MIC, we could find App were resistant to many antimicrobial agents Therefore, clinical veterinarians should only select and use antimicrobial agents effective to App to reduce the occurrence of drug resistance in bacteria.

Table 1. Distribution of serotypes according to year.

A / B	1	2	5	7	15
2012			1		
2013			1		
2014	8		4		
2015	13	3	1	1	
2016	7		2		
2017	12	2	6		7
2018	7		16		8
2019	1		1		11
Total	48	5	32	1	26

A: Serotype; B: Number of isolates

Table 2. MIC of 11 antimicrobial agents for 85 App strains isolated from pigs during 2012 to 2018.

Antimicorbial agents	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Resistance rate (%)
Ampicillin	2	512	52.94
Cefazolin	1	4	-[a]
Ceftiofur	0.25	0.25	1.18
Doxycycline	2	8	61.18
Enrofloxacin	0.25	8	45.88
Florfenicol	8	128	52.94
Lincospectin	16	32	-[a]
Tiamulin	8	16	7.06
Tilmicosin	8	16	7.06
Trimethoxazole-sulfamethoxazole	8	256	77.65
Tylosin	32	64	-[a]

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Pathological study between Swine Gastric Ulcer and *Helicobacter suis* in Central-South Region of Taiwan

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E-mail: ncyuvetlb@gmail.com

Introduction

Gastric ulcer is a common disease entity of pigs worldwide, with prevalences of up to 93 %. It can result in economic losses due to decreased daily weight gain, decreased feed intake and sudden death (1). Recently more studies showed *Helicobacter suis* (*H. suis*) was a significant factor causing gastric ulcer. Also *H. suis* is a zoonotic bacteria that can transmitted to human by close contacting to pigs and consmption of contaminated pork (2). Therefore, the purpose of this study was to evaluate the presence of *Helicobacter* spp. (*H.* spp.), *H. suis* and *Helicobacter pylori* (*H. pylori*) by PCR in swine affected by gastric ulcer in Taiwan and tried to use IHC method to examine its location for *H. suis* colonization.

Materials and Methods

Clinical cases were collected from pigs with typical gastric ulcer in the Animal Disease Diagnostic Center of National Chiayi University in Taiwan during January 2019 to January 2020. Finally, a total of 58 stomachs from 25 clinical cases were collected. In this study, we categoried these cases by macroscopic lesions first. Then we used PCR to detect three target bacteria (*H.* spp., *H. suis and H. pylori*) that can often cause swine gastric ulcer (3). And four parts of stomach including *Pars oesophagea*, fundus, body and pylori regions were collected and kept in 10 % formalin for hematoxylin and eosin (H&E) staining, silver staining and immunohistochemistry (IHC) method to analyze the prevalence and distribution of *H. suis* (4).

Results

Among the total of clinical cases, 21 cases belonged to gastric ulcer in the *Pars oesophagea*, 2 cases in the body region, and 2 cases in the pylori region. Furthermore, we found that a total of 31 stomachs from 25 cases belonged to gastric ulcer were highly positive in the *Pars oesophagea* (83.8%)(Table 1). PCR analysis of the gastric sample revealed the presence of *H.* spp. in 27/58 (46.5 %), *H. suis* in 23/58 (39.7 %) but none of the samples was positive for *H. pylori* (Table 2). The *H.* spp. positive samples were also positive for *H. suis*, while *H. pylori* was not detected. The result of silver stain and IHC showed in Figure 1.

Table 1. Macroscopic lesion of clinical cases

Parts	cases	GU stomachs	Rates (%)
Pars oesophagea	21	26	83.8
Fundus	0	0	0
Body	2	2	6.5
Pylori	2	3	9.7
Total	25	31	100

Table 2. PCR analysis.

Target	Samples (n=58)	Rates (%)
***H.* spp.**	27	46.5
H. suis	23	39.7
H. pylori	0	0

Conclusions and Discussion

The result showed that gastric ulcer of pigs were almost exclusively found in the *Pars oesophagea*. And we could detected spiral-like bacteria in lesion regions by silver staining and IHC method. PCR analysis showed the high prevalence of *H. suis* in Taiwan and it was the predominant detected in this study. In conclusion, PCR is effective for the detection of *H. suis*. By using it, we can early diagnosis and then treat immediately. Although our study did not collected too much cases, we hoped to establish a standard for Taiwan dealing with gastric ulcer caused by *H. suis* infection in the future.

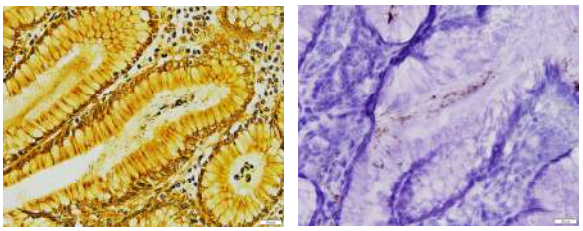

Figure 1. The results of silver staining and IHC method.

Acknowledgments

The authors thank the Animal Disease Diagnostic Center of National Chiayi University for providing us with clinical cases. We also thank Prof. H.C. Kuo and C.F. Wu for technical assistance and statistical evaluation.

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Results

Among the total of clinical cases, 21 cases belonged to gastric ulcer in the *Pars oesophagea*, 2 cases in the body region, and 2 cases in the pylori region. Furthermore, we found that a total of 31 stomachs from 25 cases belonged to gastric ulcer were highly positive in the *Pars oesophagea* (83.8%)(Table 1). PCR analysis of the gastric sample revealed the presence of *H.* spp. in 27/58 (46.5 %), *H. suis* in 23/58 (39.7 %) but none of the samples was positive for *H. pylori* (Table 2). The *H.* spp. positive samples were also positive for *H. suis*, while *H. pylori* was not detected. The result of silver stain and IHC showed in Figure 1.

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Target	Samples (n=58)	Rates (%)
***H.* spp.**	27	46.5
H. suis	23	39.7
H. pylori	0	0

Conclusions and Discussion

The result showed that gastric ulcer of pigs were almost exclusively found in the *Pars oesophagea*. And we could detected spiral-like bacteria in lesion regions by silver staining and IHC method. PCR analysis showed the high prevalence of *H. suis* in Taiwan and it was the predominant detected in this study. In conclusion, PCR is effective for the detection of *H. suis*. By using it, we can early diagnosis and then treat immediately. Although our study did not collected too much cases, we hoped to establish a standard for Taiwan dealing with gastric ulcer caused by *H. suis* infection in the future.

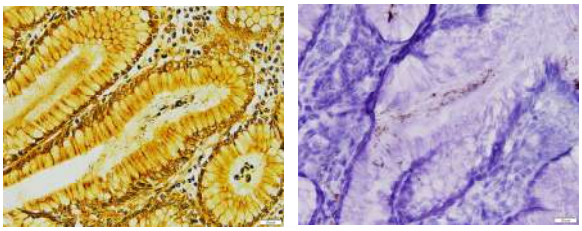

Figure 1. The results of silver staining and IHC method.

Acknowledgments

The authors thank the Animal Disease Diagnostic Center of National Chiayi University for providing us with clinical cases. We also thank Prof. H.C. Kuo and C.F. Wu for technical assistance and statistical evaluation.

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Identification of low abundance intestinal microbiome in farrowing sows using next generation sequencing

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Introduction

Next generation sequencing (NGS) is a modern diagnostic method which allows to estimate the relationship between the microbial communities. Sequencing is based on molecular technique aimed at recognizing gene, in case of bacteria - 16S rRNA.(1). NGS enables to identify a wide range of bacteria in individual taxonomic categories, for which culture-dependent methods have not been effective.(2) In most healthy pigs, the gut microbiome is dominated by bacteria *Firmicutes* and *Bacteroidetes,* being the most abundant. (4) The cut-off level is usually between 1% to 3% of identified sequences, depending on the research assumptions. In this study, we focused on less representative intestinal microbiota in sows, however it can tip the balance in an individual animal as well as their offspring.

Materials and Methods

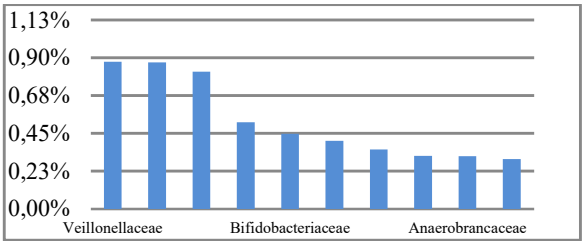
8 sows were randomly assigned to the experimental group. On the day of farrowing, fecal swabs were collected for DNA extraction. The metagenomic analysis was performed on the basis of the hypervariable region V3-V4 in the gene 16S rRNA. The NGS was performed in the sequencer MiSeq Reagent Kit v2 (Illumina) and the data analysis using software MiSeq Reporter (MSR) v2.6. For the need of this study at the subsequent taxons identification (Family, Genus and Species) "cut-of" was established at 1% and all identified DNA sequences below 1% were used for the analysis.

Results

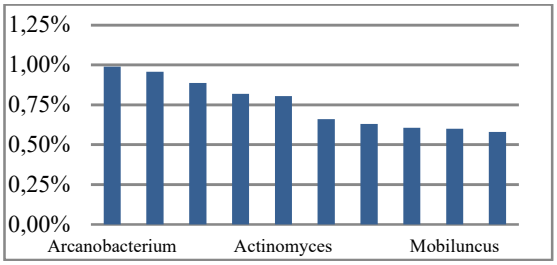
83 Families were identified at this taxonomic rank. Top ten reads (<1%) are shown in Graph 1. At the Genus level we have recognised 159 bacterial genera (<1%) and 10 are presented in Graph 2. At the Species level 273 bacteria were distinguished (<1%), top ten are presented in Graph 3.

Conclusions and Discussion

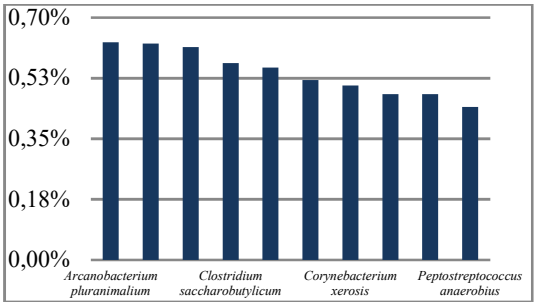
The presence of specific bacterial communities in the microbiome affects the host's organism, its resistance and the use of nutrients (3). Among top bacterial Species identified: *Arcanobacterium pluranimalium, Actinomyces hyovaginalis, Porphyromonas endodontalis, Prevotella buccalis* and *Peptostreptococcus anaerobius* - are opportunistic pathogens. The other four bacteria: *Natronincola peptidivorans, Clostridium saccharobutylicum, Prevotella copri* and *Anaerococcus tetradius* are commensals taking part in metabolic transformations. Our study suggest that microbial profiling of low-abundance microbiome may have an impact in the future on the development of some opportunistic infections. Numerous low-abundant metabolic bacteria may be incorporated as an additional point for the characterization of the pigs metabolome.



Graph 1. Top ten identified Families (cut-off <1%).



Graph 2. Top ten reads at Genus level (cut-off <1%).



Graph 3. Top ten identified Species (cut-off <1%).

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Characteristic and antimicrobial sensitivity of *Escherichia coli* strains isolated from pigletts in polish pig farms

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Introduction

PWD (post weaning diarrhoea) and ED (oedema diseases) in pigglets during weaning period, result ing from infection with pathogenic strains of *E.coli.* The microorganisms remain also important for the human health due to direct contact with infected animals as well as spreading between humans. Loses resulting from PWD and ED are currently one of the major economic problems in the global pigs production (1, 2). The extent of the problem is influenced by the presence of the microorganism, as well as environmental conditions, feeding, uncontrollable antimicrobial therapy and the lack of diagnostics (3). The goal of the study was pheno- and genotypic characterization and an assesment of their antimicrobial sensitivity of *E.coli* strains for bacteria isolated from pigletts after weaning.

Materials and Methods

In the period from January 2018, till May 2019 fecal swabs were collected on the pig farms (n=45) located throughout all over Poland. Material was obtained from pigglets (n=254) using sterile swabs and than transported on the culture medium (Equimed, Polska) at 4 ºC directly to the Diagnostic Laboratory at the Wroclaw Faculty of Veterinary Medicine. Immediately after receiving specimens were inoculated into the blood agar (Oxoid, Germany) and McConkey culture medium (Oxoid, Germany) and than incubated in 37 ºC for 2 days (48h). Obtained results were confirmed using PCR for selective amplification of *E.coli* 16S rRNA (Sabat et al., 2000). For detection of antigens : F4 (K88), O8:K87, O138:K81, O139:K82, O141:K85, O147:K89, O149:K91 and O157, the commercial multivalent serum (SSI Diagnostica, Denmark) and monovalent serum (Biomex, Poland) has been used respectivelly. Detection of Stx2e has been performed using method by Beutin et al. (2007). For identification of ST and LT toxins the method proposed by Wang et al. (2017) has been used. Antimicrobial sensitivity using disc-diffusion method was estimated on the Mueller-Hinton culture medium (BioMaxima, Lublin, Poland) with regard to the eight selected antibiotics: Vancomycin VA (BioMaxima, Lublin, Poland), Chloramphenicol C (BioMaxima, Lublin, Poland), Ampicillin AMP (BioMaxima, Poland), Gentamicin CN (BioMaxima, Poland), Tetracycline TE (BioMaxima, Poland), Erythromycin E (BioMaxima, Poland), Neomycin N (Oxoid, UK), Spectinomycin SPT (Oxoid, UK).

Results

A total of 168 *E. coli* strains were obtained in this study ; 106 were represented by clinical cases (animals with diarrhea, oedemas, fever) and 62 by pigs without any clinical manifestation. The O 149:K89 (n=3) and O 139:K82 (n=3) serotypes were found; additionally - one of them was identified as F4. The O 141 :K85 serotype was identified once. Ten strains were represented by F18, and seven by the gen responsible for ST production. No Stx2e strain has been identified.
The proportion of *E.coli* isolates exhibiting resistance to 3,7 and 8 from the all tested antibiotics was 33%, 50% and 17% respectivelly. Detailed characteristics of antimicrobial suscptibility shows table 1.

Table 1. Antimicrobial characteristics of *E.coli* strains isolated from pigglets after weaning.

Antibiotic	Inhibition zone (mm)	R*(%)	LS*(%)	S*(%)
VA	17-21	100	0	0
C	21-27	83.3	0	12,7
AMP	16-22	74.6	12,7	12,7
CN	19-26	12.7	12.7	74.6
TE	18-25	66.7	0	33.3
E	22-30	100	0	0
N	18-27	33.4	50	16.6
SPT	11-16	50	33.4	16.6

*R – resistant ; LS – Low Sensitive ; S - sensitive

Conclusions and Discussion

High antigenic diversity as well as heterogenous ability with regards to production of toxins and antimicrobial susceptibility has been found among *E.coli* strains isolated from pigglets in Polish pig farms during post weaning period. This points to the need of detailed diagnostic procerure, including antimicrobial resistance (AMR).

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Comparison of phytogenic feed additive and antimicrobial agents' effects on finisher pigs naturally infected with *Brachyspira hyodysenteriae* and *Lawsonia intracellularis*

Ching-Fen Wu[1], Hsin-Heng Chiang[1], Kai-Ti Huang[1], Che-Cheng Liao[1], Che-Wei Liao[1], Marko Vasiljević, Jasna Bošnjak-Neumüller[2], Hung-Chih Kuo[1]

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Introduction

Brachyspira hyodysenteriae is the most important causal agent of swine dysentery (SD). *Lawsonia intracellularis* is an obligate intracellular bacterium that can cause porcine proliferative enteropathy (PPE). SD and PPE are considered as important swine enteric diseases resulting in diarrhea and sudden death in grower and finisher pigs that can cause severe economic losses in pig production (1). As the drug resistance of antimicrobial agents (tiamulin and lincomycin) is becoming an issue on farms (2), other approaches are needed to address the intestinal problems in pigs without using antimicrobial drugs. The aim of this trial was to assess and compare the efficacy of antimicrobial agents and a commercially available phytogenic feed additive (PFA) on finisher pigs by using quantitative polymerase chain reaction (qPCR) to quantify bacteria numbers.

Materials and Methods

The trial performed on a commercial wean-to-finish swine farm, lasted 7 weeks and included 800 20-week-old pigs divided into 2 groups: trial group and control group. Each group was fed with basic diet. The Trial group was also fed with feed containing 2 kg/ton of commercial PFA (PATENTE HERBA® PLUS, PATENT CO. DOO, Serbia), until the end of the trial. The Control group was fed with 2 kg/ton of tiamulin until 33rd day and 2 kg/ton lincomycin from 33rd to 45th day of the trial. Fecal floor samples (n=288) were collected using gauze wetted by phosphate buffered saline (PBS), while anal swabs (n=168) were collected using cotton swabs on days 0, 7, 14, 21, 28, 35, 42 and 49. The numbers of *B. hyodysenteriae* and *L.intracellularis* bacteria in fecal samples and anal swabs were determined by qPCR (3,4). Clinical signs and fecal scores of pigs were recorded every week. At the end of study, intestinal lesion scores in both groups were calculated.

Results

qPCR analysis of fecal samples and anal swabs are shown in Figure 1. At the end of the trial, the DNA copies of *B. hyodysenteriae* and *L. intracellularis* in both type of samples in both groups decreased and there were no statistical differences between groups during 0-6 weeks of the trial. In the last week of the trial, the numbers of *L. intracellularis* in both type of samples increased in the control group, while in the trial group declined. The intestinal lesion scores are listed in Table 1.

Conclusions and Discussions

The results demonstrated that the commercial PFA as a feed additive was able to reduce the numbers of *B. hyodysenteriae* and *L. intracellularis* in swine stools and anal swabs of finishing pigs. In comparison with the time when there was an outbreak of SD and PPE, at the end of the trial, the pigs in the trial group showed no obvious clinical signs, and lower fecal scores. Thus, the commercial PFA was as effective as antimicrobial agents in preventing and controlling the outbreak of SD and PPE. Possibly the withdrawal of antimicrobial agents in last week of the trial, led to higher numbers of bacterial DNA copies in control group in comparison to the trial group. In conclusion, the commercial PFA can be an alternative when dealing with SD and PPE and until pigs reach market age.

Table 1. Intestinal lesion scores in both groups.

Group	Jejunum	Ileum
Trial group	0	0
Control group	0	4

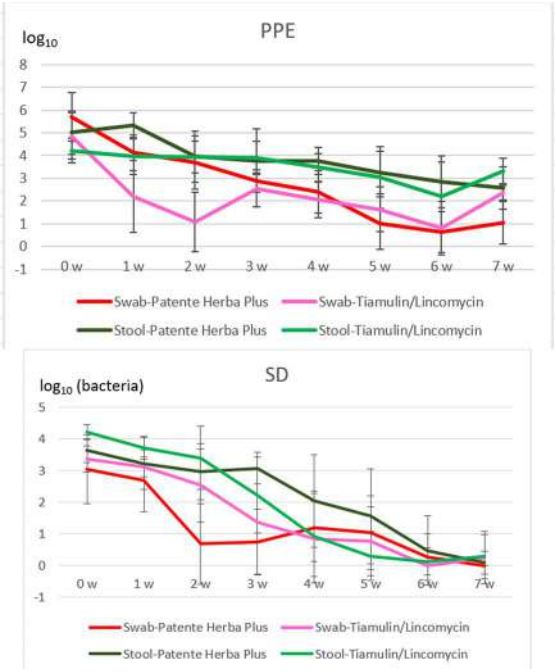

Figure 1. The result of qPCR.

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Ching-Fen Wu[1], Hsin-Heng Chiang[1], Kai-Ti Huang[1], Che-Cheng Liao[1], Che-Wei Liao[1], Marko Vasiljević, Jasna Bošnjak-Neumüller[2], Dan-Yuan Lo[1], Hung-Chih Kuo[1]

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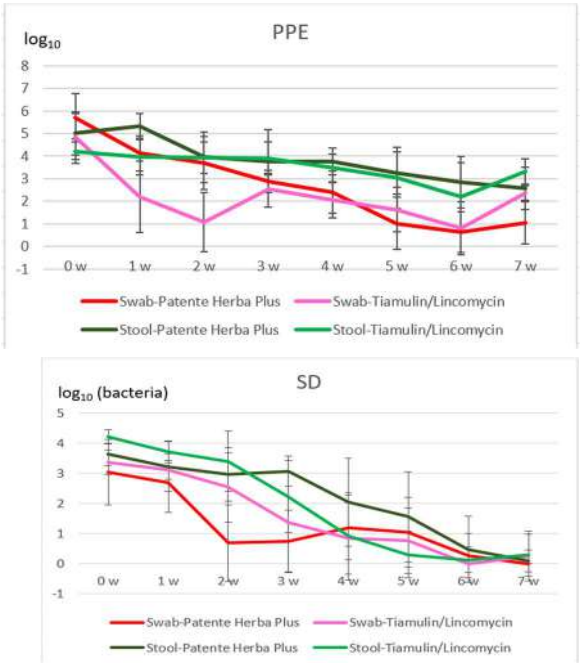

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Design and validation of a Differentiating Infected from Vaccinated Animals Quantitative Polymerase Chain Reaction (DIVA qPCR) for *Lawsonia intracellularis.*

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Introduction

Lawsonia intracellularis in pigs is associated with two clinical manifestations - porcine intestinal adenomatosis (PIA) and proliferative haemorrhagic enteropathy (PHE), or infections can remain subclinical (1-3). Laboratory methods that can differentiate infected from vaccinated animals (DIVAs) have been developed for other pig pathogens, such as pseudorabies virus, classical swine fever virus, foot and mouth disease virus and *Salmonella* (4-7). However, currently, there are no markers for the commercially available *L. intracellularis* vaccines.

Based on the analysis of the complete sequences of four *L. intracellularis* strains, including two wild type (WT), Enterisol® Ileitis vaccine and its parental strain, we have now developed a Differentiating Infected from Vaccinated Animals Quantitative PCR (DIVA qPCR). This allows the Enterisol® Ileitis vaccine strain to be discriminated from WT *L. intracellularis* shed in faeces, and respective proportions determined.

Materials and Methods

Assembly using Prokka and alignment of four different *L. intracellularis* strains (Enterisol® Ileitis strain, its parental strain and two publicly available sequences) was performed aiming to find differential markers for Enterisol® Ileitis. This analysis gave us potential differential genes from which we chose *gene 1,* a gene that harbours a deletion that occurs in the Enterisol® Ileitis strain. Primer and probe design were designed based on the known conserved *aspA* gene and a differential gene (*gene 1*) (Figure 1). The DIVA qPCR performance was compared with other published *L. intracellularis* PCRs. Finally, faecal samples from an animal study, as well as known positive and negative samples, were tested.

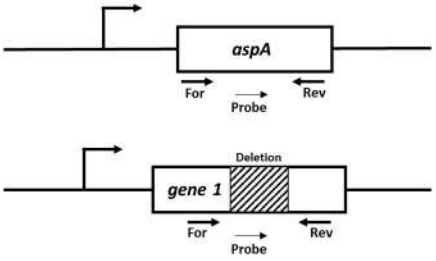

Figure 1. DIVA qPCR primers and probes design.

Results

We were able to differentiate accurately *L. intracellularis* Enterisol® Ileitis vaccine strain from other WT *L. intracellularis* strains based on the differential *gene 1* and *aspA* (Table 1).

Discussion and Conclusions

Probe based qPCR was the chosen method for differential detection between Enterisol® Ileitis vaccine and WT *L. intracellularis* strains. This approach allowed the detection of multiple targets simultaneously in each sample. In this study, two reactions took place in each reaction tube, one detecting *aspA* (a gene known to be conserved in *L. intracellularis*) and the differential target *gene 1*. The quantity of Enterisol® Ileitis strain was calculated by the difference of total *L. intracellularis* (given by the *aspA* gene) minus the amount of complete *gene 1*, that in case of a wild type infection, gives the same copy number as calculated with *aspA*.

Table 1. Example of ΔCt values and copy numbers of each type of sample that can be found in the field.

	ΔCt		*L. intracellularis* copies		
SAMPLE	***aspA***	***gene 1***	**Total *aspA***	**Total *gene 1***	**Enterisol® Ileitis**
WT	35.51	36.02	1.85E+05	2E+05	-
Vaccinated	23.11	NA	2.85E+08	-	2.85E+08
Mixed	27.64	32.95	1.95E+07	1.20E+07	7.50E+06

This new DIVA qPCR allows us to detect if WT *L. intracellularis* infection is occurring after an Enterisol® Ileitis vaccination.

Acknowledgments

We thank Dr S Fitzgerald for his suggestions and critical review during the project. We also acknowledge Boehringer Ingelheim Vetmedica GmbH for the Enterisol® Ileitis vaccine and parental sequences and the funding of this project.

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Molecular Diagnosis of VEROTOXIN 2E (Edema Disease) in Brasilian pig breeding

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Introduction

Edema Disease (ED) is caused by pathogenic strains of *Escherichia coli* (VTEC) that colonise the small intestine of piglets between 6-7 and 15-16 weeks of age. They can multiply and produce an active protein called Verotoxin 2e (Vt2e) (1, 4). It is a highly lethal disease, which causes high mortality and a large number of runt piglets (2). A study on the prevalence of Vt2e in healthy or unhealthy pigs provides evidence for its presence in countries in Europe, North America, South America, Asia and Africa (3). The diagnosis of Vt2e in a bacteriological culture from the intestine, which gets costly and time consuming compared to the qPCR of oral fluid, which proves to be an efficient and fast alternative method (5). This study demonstrates the distribution of Vt2e in 3 different regions in Brazil, by detecting the Vt2e gene in swine oral fluid, in 2018 and 2019.

Materials and Methods

A total of 229 swine oral fluid samples (Figure 1) were analysed between 1 and 18 weeks of age, form both the nursery and fattening phases. The analysis comprised 49 farms from 3 different regions in Brazil (South, South-east and Midwest), during 2018 (14 farms) and 2019 (35 farms), at the DIAGNOS-Hipra Saúde Animal Laboratory (Brazil). For the diagnosis of the Vt2e gene, oral fluid was collected by means of the VEROCHECK kit and preserved in Watman® FTA ELUTE. The protocol developed by Hipra (5) was used for the extraction of bacterial DNA and the molecular qPCR technique.

Figure 1. Oral fluid sample procedure: pigs chewing a cotton rope.

Results and Discussion

Out of the 72 samples received in 2018 from 14 farms, 67% proved to be positive for Vt2e, and all of farms were positive. In 2019, out of the 157 samples received from 35 farms, 59% proved to be positive, and 86% of total farms were positive (Figure 2). The Vt2e gene was diagnosed with a higher prevalence in the stages between the nursery and the beginning of the fattening period, from 1 to 13 weeks of age, which corroborates the previous data (3) (Figure 3).

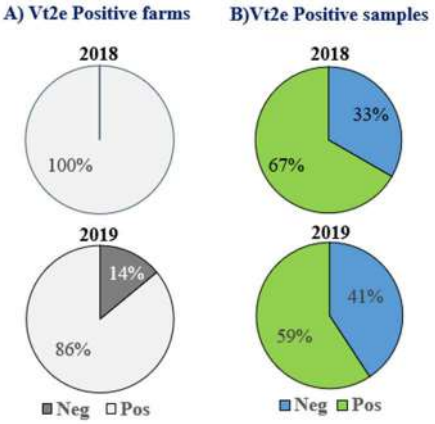

Figure 2. Percentage of positives and negatives for Vt2e in the farms (A) and samples (B) analysed between 2018 and 2019 in Brazil.

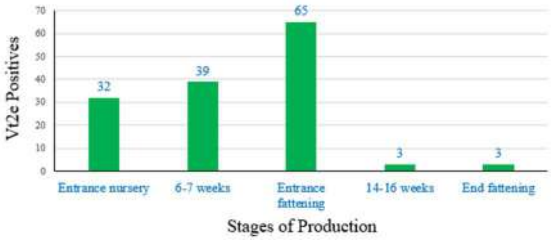

Figure 3. Prevalence of positive cases in all samples from the farms tested at different stages of piglet growth in 2018 and 2019.

Conclusion

The data from this paper confirms the presence of the Vt2e gene in pigs of different ages from farms located in 3 different Brazilian regions.
The diagnosis of the gene by qPCR proved to be efficient and less stressful to the animals.

Acknowledgements

The authors would like to thank the Hipra swine commercial team for their technical support.

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Study of *Bordetella bronchiseptica* and Analysis on Atrophic Rhinitis from diseased swine in Taiwan

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Introduction

Atrophic rhinitis (AR) is a contagious respiratory disease of pigs that is highly prevalent throughout the world. In addition, it is a difficult problem to deal with especially in nursery pigs. There are two forms of AR that have been recognized in terms of the causal agents. Nonprogressive form with mild to moderate turbinate atrophy is induced by *Bordetella bronchiseptica* (BB). Nevertheless, diseased pigs infected with toxigenic isolates of *Pasteurella multocida* (PM) in combination with BB have more severe signs, such as epistaxis, head tilt and twisted snout. There are no reports regarding BB isolation in Taiwan and the last survey and investigation on AR was conducted more than twenty years ago. Therefore, the aim of this study is to provide an update on current conditions by AR investigating diagnostic samples from clinically diseased pigs in Taiwan. On the other hand, this study also aimed to show the correlation between AR lesion grades and lung damage through the statistical analysis of turbinate and lung lesion ratios.

Materials and Methods

600 clinically diseased pigs from a total of approximately 50 pig farms were studied. Visual scoring of the nasal turbinate bones was performed on vertically sectioned at the level of the first premolar teeth and was examined in a manner for turbinate bone atrophy (TA), and nasal septum deviation (NSD). Each of the four scrolls of the ventral turbinate bones was scored according to the European Pharmacopoeia guideline [1]. TA and NSD scores were summed for each individual to a maximum value of 18 (nasal lesion score, NLS). NLSs were divided into three intervals which could be defined as the patterns of AR, including 0-4, mild, 5-8, moderate, 9-18, severe. For this study, a total of 33 bacterial isolates were collected from diseased pigs as mentioned above. The bacteria examined were 20 isolates of BB, 24 isolates of PM. 150 affected lungs were selected from diseased pigs to calculate lung lesion ratios through an objective method that allows the quantification of the affected lung area (%) by means of a picture and the use of the corresponding software (ImageJ®) [2]. Spearman's rank correlation coefficient were used to analyze the relationship of these two variables.

Results

Our results demonstrated that the average NLS was 5.98 with 16.50% septum affected. All visual scorings were distributed between 0-18 scores. And based on the data, further analysis were illustrated as Fig 1. In this study, results of NLS and lung lesion ratios shown as Fig 2. Spearman's rank correlation coefficient value (r_s) was 0.496, which is moderate concerning the relationship between two variables.

Conclusions and Discussion

Based on the findings in this study, the situation of AR infection appeared to be common among swine industry in Taiwan, although diseased pigs show no clinical signs or obvious lesions such as head tilt and twisted snout. Furthermore, diseased pigs with AR had severe lung lesions. These correlation data showed that preventing pigs from AR tended to be an effective control of respiratory conditions. Patterns of AR depends on the range of NLS.

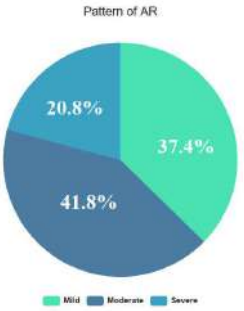

Fig 1.The proportion of patterns of AR from clinical samples in this study.

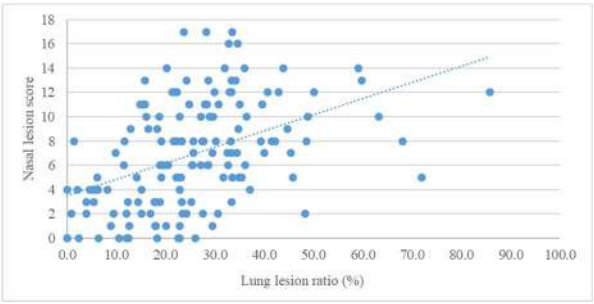

Fig 2. Scatter chart of NLS and lung lesion ratio. r_s = 0.496, $|r_s| \leqq 0.39$: weak concerning correlation; $0.4 \leqq |r_s| \leqq 0.59$: moderate concerning correlation; $0.60 \leqq |r_s|$: strong concerning correlation.

Acknowledgments

This work was supported by Animal disease diagnostic center, National Chaiyi University, Taiwan. Sincerely, the author thanks colleagues in lab for technical assistance.

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Genetic diversity of porcine hemothropic mycoplasmas in Brazil

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Introduction

Porcine hemotropic mycoplasmas are small bacteria lacking a cell wall that show tropism by the red blood cells of several hosts, including pigs (1). To date, three species, namely *Mycoplasma suis*, *Mycoplasma parvum* and *Mycoplasma haemosuis,* have been described as porcine hemoplasmas (PHs). While *M. suis* has been reported all over the world and associated to infectious anemia in pigs (2), *M. parvum* is a non-pathogenic hemoplasma reported in a few countries (3, 4). At the genomic level, there is no report of genetic diversity associated to PHs, even though they share many characteristics (1). The present study aimed to investigate the genetic diversity of porcine hemoplasmas detected in domestic pigs from commercial farms in Brazil.

Materials and Methods

Blood samples from 450 pigs were collected at the time of slaughter. The animals originated from 30 different farms located in Goiás State, Midwestern Brazil. DNA extraction followed a previously described protocol (5). Conventional PCR (cPCR) for the *gapdh* gene was performed in order to avoid false negative results on quantitative real-time PCR (qPCR) (6). *gapdh*-positive DNA samples were submitted to qPCR assays targeting a fragment of PHs-16S rRNA gene (7). Samples with quantification values higher than 10^5 copies/µL were tested for a cPCR targeting PHs-16SrRNA gene (8). Positive samples on the previous test were cloned following the manufacturer's recommendations, and sequencing was performed according to Sanger's method. Genetic diversity was analyzed through DNASP software, and phylogenetic analyses by the Bayesian method.

Results and Discussion

Porcine hemoplasmas DNA was detected in 68.89% of the samples, and all farms had at least one positive animal, indicating a high occurrence of PHs in finishing pigs from commercial herds in Brazil. Five of the 33 samples presenting quantification values between 10^5 and 10^6 copies/µL were cloned, resulting in 22 sequences. Blast analyses indicate that the sequences obtained from five animals, in five different farms, had a higher identity to *M. parvum* than *M. suis* sequences previously deposited in Genbank.

Nucleotide diversity analyses of the 22 cloned 16S rRNA sequences resulted in seven genotypes and nine variable sites, from which four pig blood samples had at least two different genotypes each. Genotype #2 was the most prevalent, being detected in four of the five pig blood samples (**Table 1**). It is argued that nucleotide substitutions may correspond to evolutionary changes, which can, in turn, generate new genotypes and species (9).

Table 1. Genotype identification of *M. parvum* 16S rRNA sequences.

		Samples				
gene	**genotype #**	**129**	**258**	**292**	**371**	**411**
16S	1	-	-	(3)	-	-
	2	(4)*	(3)	-	(2)	(4)
	3	-	-	-	(2)	-
	4	-	-	-	-	(1)
	5	(1)	-	-	-	-
	6	-	-	(1)	-	-
	7	-	-	-	(1)	-

*Number inside parentheses ( ) represent the total of sequences representing the same genotype.

Phylogenetic analyses demonstrate that the 16S rRNA cloned sequences formed a large clade, closely related to *M. parvum* detected in Brazil, China and Japan, where two clusters have been described, one close to *M. suis* and another close to *M. parvum* (3, 4, 9). Even though *M. suis* was not detected in our study, it is possible that this pathogen circulates among domestic pig herds in Brazil.

Conclusions

This study demonstrates, for the first time, genetic diversity associated to *M. parvum* infection, and the occurrence of different genotypes in a single animal and between commercial pig farms in Brazil.

Acknowledgments

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European survey on lung lesions in slaughter pigs

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Introduction

Lung lesions scoring at slaughterhouses provide valuable information about the respiratory health in the pig population. Lesions suggestive for previous *M.hyo* or *A.p.* infections and their scoring were described before. Scoring of those lesions allows quantifying the problems with enzootic pneumonia end pleuropneumonia. The aim of this survey was to collect the results of lung scoring performed in most of swine producing European countries in 2019.

Materials and methods

Ceva Lung Program scoring methodology was implemented to score the lesions at the slaughterhouse. The results were collected from 20 European countries in the 12 months period from December 2018 till end of November 2019. The mean values and quartiles were calculated for % of lungs with bronchopneumonia (%BP), % of affected lung parenchyma out of sick lungs (% parenchyma), % of dorso-caudal pleurisy (%DP) and APP index (APPI). For the two latter indicators the results from France were not included, because there they were not scored routinely.

Results

The total number of scored lungs was 425058 from 3566 reports with the average of 119 lungs per batch. The median value of %BP was 40,37% with the Q1=19,83% and Q3 63,10%. The median of affected parenchyma was 5,51% with the Q1=2,86% and Q3=8,83%.

Table 1. Distribution of values related to EP-like lesions

	% of lungs with EP-like lesions	% of affected lung parenchyma	% of lungs with scars
Q1	19.83%	2.86%	2.28%
Median	40.37%	5.51%	7.31%
Q3	63.10%	8.83%	15.14%

For % DP the median, Q1 and Q3 were 9,88%; 3,48% and 23,55% respectively and for APPI the corresponding values were 0,42; 0,18 and 0,87 respectively.

Table 1. Distribution of values related to A.p.-like lesions

	Percent of Dorsocaudal Pleurisy	APPI Index
Q1	3.48%	0.09
Median	9.88%	0.26
Q3	23.55%	0.61

Conclusions

The results of this survey conducted in 20 European countries in 2019 demonstrated very similar distribution of the values as the previous year 2018. With the almost 4% more lungs scored in 2019, those results confirm the value of CLP as a repeatable scoring methodology. The values of A.p. like lesions are worse than in 2018, which may be related to the continuous decrease of the use of antimicrobials in European swine herds.

Assessment of porcine lung lesions at slaughter from batches immunized with different conventional *Mycoplasma hyopneumoniae* vaccines and unvaccinated animals

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Background and Objectives

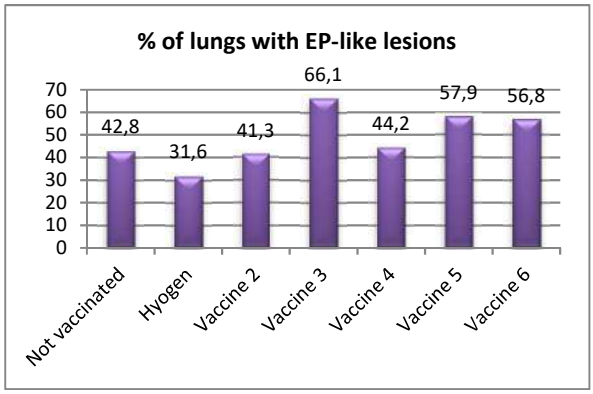
Mycoplasma hyopneumoniae (MH) is the primary agent of enzootic pneumonia (EP), which in case of viral co-infections can lead to the Porcine Respiratory Disease Complex. Vaccination against MH is applied worldwide to control possible infections. The goal of this study was to compare lung lesions at slaughter from fattening pigs vaccinated with six different conventional MH vaccines or unvaccinated.

Material and Methods

Slaughter lung checks were performed in Germany and Austria from 2017 until 2019, for 19238 lungs from 174 batches. Lungs were assigned to each one of the following groups according to the MH vaccine and protocol used: unvaccinated piglets (0), Hyogen® (1), single-shot (SS) 2ml vaccine (2), SS 2ml vaccine (3), combined M.hyopneumoniae + PCV2 (COM) 2ml vaccine (4), COM 2ml vaccine (5) and SS 1ml vaccine (6). The prevalence of bronchopneumonia (BP) and % of affected lung surface (LS) was calculated and compared among groups.

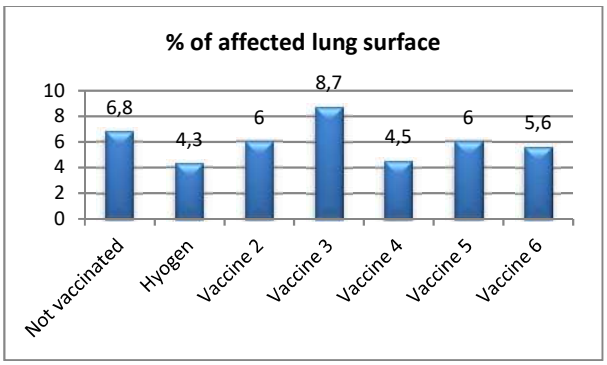
Results

BP% for each of the seven groups was, 0: 42.8%, 1: 31.6%, 2: 41.3%, 3 :66.1%, 4 :44.2%, 5 :57.9%, 6 :56.8%. BP% was negatively associated only with group 1 compared to unvaccinated animals, although the association was non-significant ($p>0.05$). Regarding comparison among vaccine groups, BP% was positively associated ($p<0.05$) with each of the other MH vaccines compared to group 1.



Graph 1. Prevalence of bronchopneumonic lesions (BP%)

In terms of LS, group 1 had numerically lower values than all other groups and significantly lower ($p<0.05$) compared to 0 (4.3% vs. 6.8%) and 3 (4.3% vs 8.7%).



Graph 2. Extension of the lesions.

Conclusion

In this study, lungs from pigs vaccinated with Hyogen® showed superior lung health in terms of EP lesions compared to unvaccinated animals and the rest of MH vaccines. However, unvaccinated animals did not perform worse than most MH vaccine groups, probably due to the high respiratory health status of those herds, managed through other means than vaccination.

Comparation of Lung Lesions in the slaughterhouse using different 1-dose and 2-dose vaccination protocols for *Mycoplasma hyopneumoniae*, in Brazil.

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Introduction

The Enzootic pneumonia, caused by *Mycoplasma hyopneumoniae* (Mhyo) has a worldwide impact on intensive swine production. The disease causes substantial losses due to decreased animal performance, higher antimicrobial use and increased susceptibility to secondary respiratory agents (1).

It is generally accepted that control of Mhyo is critical for reducing economical losses in infected swine farms. One of the important tools used for this is vaccination (2). Different vaccination protocols are adopted and their differences based on impact on production are measured.

The aim of this study was to compare the levels of lung lesions in vaccinated animals with various Mhyo protocols.

Materials and Methods

558 batches were involved in the analysis, totaling 51,774 lungs scored in the period of 2019. These batches came from the main pork-producing states in Brazil. The lungs were scored at the slaughterhouse, using the Ceva Lung Program (CLP). The parameters measured were: EP Index, percent lungs with Ep-like lesions and percentage of cranial pleurisy.

Results

The results are shown in table 1. Animals vaccinated with 1-shot Mhyo vaccine, presented 1.97 of EP Index, 52% of Bronchopenumonic lungs, 5.43% of Affected surface of pneumonic lung and 2.55% of Cranial Pleurisy. While animals vaccinated with 2-shot Mhyo vaccine presented 2.77 of PE Index, 65% of Bronchopenumonic lungs, 6.38 % of Affected surface of pneumonic lung and 3.36% of Cranial Pleurisy.

Table 1. EP-like lesions of 51,774 lungs scored in Brazil.

	# Batches	# Animals	*EP Index*	Bronchopneumonic lungs (%)	Affected surface / pneumonic lungs (%)	Cranial Pleurisy scoring (%)
1 shot	454	42412	1.97[a]	52.12[a]	5.43[a]	2.55[a]
2 shots	104	9362	2.77[b]	65.7[b]	6.38[b]	3.36[b]
P Value			**P<0,05**	**P<0,05**	**P<0,05**	**P<0,05**

Conclusions and Discussion

EP-like lesions have relatively high prevalence in lungs from Brazil farms which, however corresponds to the numbers described in other studies (3,4).

A positive effect on animal lung health can be achieved through 1-dose Mhyo vaccination, when compared to animals vaccinated with 2 doses of Mhyo vaccines.

The decrease in lung lesions shows a strong correlation in the animals performance(5).

The use of a 1-dose vaccine for Mhyo is an excellent alternative for the control of this agent, improving respiratory health and thus helping to increase profitability, resulting from better animal performance.

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Results of lung lesion scoring in Brazil, Argentina and Colombia in 2019

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Introduction

The scoring of lung lesions in pigs for slaughter provides very important information on respiratory health in the swine population(1). The score of these lesions allows quantifying problems with enzootic pneumonia and pleuropneumonia, in addition to providing an epidemiological survey of these diseases' situation in the regions studied. The objective of this survey was to collect the results of lung score performed in three important Latin American pig-producing countries in 2019.

Materials and Methods

Ceva Lung Program (CLP) scoring methodology was implemented to score the lesions at the slaughterhouse. The results were collected from 3 Latin American countries from January to December 2019.
The median values and quartiles were calculated for % of lungs with EP-like lesions, % of affected lung parenchyma, % of lung with scars, % of dorso-caudal pleurisy (%DP) and APP index (APPI).

Results

The total number of scored lungs was 181.869 from 2.565 reports with the average of 85 lungs per batch. The median value of % of lungs with EP like lesions was 51.9% in Argentina, 57.84% in Brazil and 58.33% in Colombia. The median of affected parenchyma was 4.05% in Argentina, 2.98% in Brazil and 3.99% in Colombia. And the % of Scars was 10.17% in Argentina, 7% in Brazil and 4.21% in Colombia. All data are shown in table 1.
And the results of the lesions suggestive of A.p. are illustrated in table 2.

Table 1. Distribution of the values regarding the EP-like lesions in lungs (expressed quartile 1 – median –quartile 3)

	% of lungs with EP-like lesions (Q1 - **M** - Q3)	% of affected lung parenchyma (Q1 - **M** - Q3)	% of lungs with scars (Q1 - **M** - Q3)
Argentina	38.91% - **51.9%** - 62.68%	1.81% - **4.05%** - 4.45%	3.90% - **10,17%** - 19.32%
Brazil	40.24% - **57.84%** - 72.49%	1.51% - **2.98%** - 5.25%	3.66% - **7.00%** - 11.00%
Colombia	32.73% - **58.33%** - 73.33%	1.32% - **3.99%** - 7.49%	1.43% - **4.21%** - 8.33%

Table 2. Distribution of values related to A.p.-like lesions (expressed quartile 1 – median-quartile 3)

	% of lungs with dorso-caudal pleurisy (Q1 - **M** -	APPI (Q1 - **M** - Q3)
Argentina	3.03% - **6.54%** - 14,26%	0.09 - **0.20** - 0.42
Brazil	2.50% - **6,06%** - 9.67%	0.08 - **0.21** - 0.3
Colombia	1.79% - **5.45%** - 10.20%	0.05 - **0.16** - 0.31

Conclusions

The data set from 3 Latin american countries in 2019 shows very similar distribution of the values as the previous studies (2,3,4). Adding the results of this survey with the previous studies, it is possible to identify the CLP as an important tool to estimate the prevalence of enzootic pneumonia and pleuropneumonia. With the creation of a significant database, it is possible to generate comparative studies between regions, countries and farms, contributing to a very consistent epidemiological study.

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Assessment of the situation of the Porcine Enzootic Pneumonia and Porcine Pleuropneumonia in COLOMBIA using Ceva Lung Program

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Introduction

The evaluation of Cranio Ventral Lung lesions (CVLL) in pigs at the slaughterhouse is a good indicator to estimate the prevalence of Enzootic Pneumonia in the farms (3). Likewise dorsocaudal pleurisy is a finding associated with Pleuropneumonia disease. Several authors have described the relation between lesions found in lung tissue and performance losses in the grow-to-finish phase1,2 (5). As with pneumonia lesions, pleurisy is also indicative for zootechnical losses in the finishing phase 3. A scoring method was previously described including an application developed for tablets that allows faster data collection of slaughter-check evaluations and its correct processing and storage - Ceva Lung Program -CLP (4). This study aims to describe the prevalence of bronchopneumonia (Enzootic Pneumonia) and dorsocaudal pleurisy lesions (Pleuropneumonia) in Colombia.

Materials and Methods

Between January and December 2019 (12 months), 1665 slaughter follow-ups were carried out (25 to 120 pigs per batch). In total 93.572 lungs were scored from 326 farms distributed in the main swine producing regions of the country. All farms vaccinate against M. hyopneumoniae and use different vaccination protocols. The lungs were analyzed using Ceva Lung Program.

Results

The results obtained are summarized in Table 1.

The mean prevalence of bronchopneumonia in pigs for region was 53,4% (range between region 42,4% - 62,4%) of slaughtered animals. The mean affected lung area by EP-like lesions was 7.3% (range per region 5,4 - 8,6%). The percentage of scarring was 7.3% (range between region 5,2 – 9,1%). The mean APP index (APPI) was 0.27 (range between region 0,2 to 0,3) and the prevalence of dorso-caudal pleurisy was 16,19% (range between region 10,64 – 40,34%).

Table 1. Percentage of lung lesions in Colombia between Jan to Dec 2019

	REGION				
	ANTIOQUIA	CENTRO	EJE	VALLE	Total
Number of CLP	290	373	347	655	1665
Numer of lungs evaluated	13786	15017	16730	44466	89999
% bronchoneumonic lungs	42,4%	52,2%	62,4%	54,2%	53,4%
% lungs lesion area	5,4%	7,0%	8,6%	7,5%	7,3%
% Scar Scoring	9,1%	5,2%	6,2%	8,3%	7,3%
Madec Index	1,75	2,38	3,21	2,56	2,50
% Dorsocaudal Pleurisy	10,64%	7,65%	40,34%	10,72%	16,19%
App Index	0,28	0,20	0,29	0,30	0,27

Conclusions

The results obtained from scoring of bronchopneumonia lesions suggestive for previous infection by Mycoplasma hyopneumoniae and dorsocaudal pleurisy, suggestive for Actinobacillus pleuropneumoniae infection, are similar to those reported in other countries of the Latino America region (1,2). The study shows a great opportunity to improve control measures in the control of respiratory diseases in Colombia. The CLP proved to be an effective tool in the evaluation and later categorization of lung lesions in pigs at slaughter.

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Economical impact of lung consolidation lesions on finishing pigs in Brazil

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Introduction

Swine respiratory diseases cause considerable decrease in production and economic impact in the swine industry. Evaluating lung lesion scores is an effective way of assessing respiratory health and the efficiency of disease control programs in swine farms. The objective of this study was to assess the economical impact caused by lung consolidation lesions on finishing pigs from Brazil.

Materials and Methods

This prospective field study was conducted in wean-to-finish pigs from a single sow farm, in the southeast of Brazil, between December 2018 and March 2019. A total of 486 pigs were individually weighed at the 75 and 180 days, to assess the individually average daily gain (ADG) through the period. The lungs of each pig were examined and scored using the Ceva Lung Program**.** Based on severity of lung lesions the animals were classified into four groups: Group 1 – pigs without macroscopic lesions (n=153); Group 2 – 0,1-5% of lesions (n=207); Group 3 – 5,1-15% of lesions (n=96); Group 4 – >15% of lesions (n=30). The economical analysis was performed through a simulation, using productive parameters and the average production costs of the study population. Productive parameters: average days on feed (105 days/group), mortality (3.5% of pigs placed), ADG (kg/day). The production costs included in the analysis were: price of weaned pigs ($11.33/pig placed), price of diet ($0.20/kg of feed), animal health vaccine costs ($0.81/pig placed), animal health antimicrobial costs and labor and farm management ($2.39/pig placed). The market pig price used was according to 2019 October in Minas Gerais state, in this case U$1.24 (Cepea/Usp – 2019).

Table 1. Productive parameters used in economical analysis.

Productive parameters		
Groups of lesions	Average of ADG (kg/day)	Average weight at the beginning of the finishing (kg/pig)
1	0.912	51.702
2	0.899	51.725
3	0.898	51.516
4	0.86	51.686

Results and discussion.

Lung lesions are often associated with declines on productivity and may indicate problems affecting animal welfare, being caused by metabolic factors that contribute to reduced growth rate, feed conversion efficiency and quality in carcasses (1,2,3,4). Animals with more lesions, showed a lower weight and consequently a lower price at the slaughter, compared with pigs without lesions, as shown in Table 1. The presence of lesions is related the decrease all productive potential, thus causing financial loss, generating impacts in the production system (5).

Table 2. Results of the economical analysis based on the relationship between the values of ADG and lung lesions score of the 333 animals included in the study.

Groups of lung lesions		Comparisons between the groups of lesions	Benefit of intervention ($/pig)
1 (0%)	**2 (0.1 - 5%)**	1x2	1.63
	3(5.1– 15%)	1x3	1.87
		1x4	6.55
		2x3	0.37
4 (>15.1%)		2x4	4.95
		3x4	4.67

Conclusions

The economical analysis confirmed that lung consolidation lesions, correlates to economic losses for the producers, because of the lower weight of the animals at slaughter or the longer time required to reach the weight.

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A survey of Asian respiratory health in finishing pigs at slaughter age

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Introduction

Lung scoring at the slaughterhouse is a valuable tool for assessment of the respiratory health status of a large number of animals at a single visit, at relatively limited cost. Different from post-mortem investigation, it assesses lung health across the whole batch of animals. Moreover, a clear relation between lung lesions present at slaughterhouse and economic impact of respiratory disease has been reported [1], making lung scoring an attractive tool for decision making and effect monitoring of veterinary interventions.

To facilitate efficient and hygienic lung lesion scoring at slaughterhouses, Ceva provides a scoring methodology Ceva Lung program (CLP). A tablet-based software tool allowing for rapid recording of the results and their processing is a part of CLP.

Materials and Methods

In between January 2018 and Jan 2020, a total of 1,157 batches of pigs (47,501 animals) were scored at time of slaughter, using CLP. Lung scorings were performed in China(26),Cambodia (4),Japan(1), Malaysia (14), Philippines (268), South Korea (44), Taiwan (30), Thailand (387) and Vietnam (383).

Lungs were scored following the CLP method [2], with presence, type and extension of lung lesions described by:

- Enzootic pneumonia (EP)-like lesions following a modified Madec methodology.
- Cranio-ventral pleurisy, to describe EP-associated secondary pleurisy.
- Scarring, describing prevalence of fissures associated with older EP-like lesions.
- Dorsocaudal pleurisy, to describe *Actinobacillus pleuropneumoniae* (APP)-like lesions
- Actinobacillus pleuropneumoniae Index (APPI), using prevalence and grade of dorsocaudal pleurisy (scale 0-4).

Results

Results for the Asian region are presented in Table 1 and 2 using percentiles (P_{25} -median - P_{75}).

Table 1. EP-like lesions

	P_{25}	Median	P_{75}
Prevalence bronchopneumonia	51.8%	72.5%	86.7%
% lung surface with bronchopneumonia	2.5%	5.3%	9.7%
% Cranio-ventral pleurisy	3.3%	12.9%	31.1%
Scars	0.0%	4.0%	16.7%

Table 2. APP-like lesions

	P_{25}	Median	P_{75}
% Dorsocaudal pleurisy	6.4%	14.0%	29.9%
APPI index	0.13	0.36	0.77

Conclusions and Discussion

Results clearly indicate there is room for improving the respiratory health of finishing pigs in the sampled countries. Both lesions associated with *M.hyopneumoniae* and *A.pleuropneumoniae* have a high prevalence. While the data by country (not shown) suggests some differences between countries exist, these have to be interpreted with caution as farm selection was not randomized. Nevertheless, the distribution reported above could be a useful tool for interpretation of lung lesion scoring results, as well as for setting targets for farms aiming for improvement of their respiratory health.

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A survey of Porcine Enzootic Pneumonia and Porcine Pleuropneumonia lesions at slaughterhouses in Thailand using Ceva Lung Program

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Introduction

Porcine Enzootic Pneumonia and Porcine Pleuropneumonia, which are caused by *Mycoplasma hyopneumoniae* (M. *hyo*) and *Actinobacillus pleuropneumoniae* (A. *p*) infections respectively, are two major contributors to the respiratory diseases in swine industry [1, 2]. These diseases result in economic loss including increased mortality, decreased growth rate, increased feed conversion ratio. Lung lesions scoring is a useful tool to quantify the problem of these diseases. Ceva Lung Program (CLP) is an innovative approach for assessment of lung lesions score [3]. The objective of this study was to survey the presence of Porcine Enzootic Pneumonia and Porcine Pleuropneumonia lesions in Thai pigs at slaughterhouses using CLP

Materials and Methods

224 batches of pig, total 11,459 lungs were scored using Ceva Lung Program at the time of slaughter. Pneumonia is scored and calculated consider the proportion of each lobe of the lungs [4]. Pneumonia scores are described as percentage of bronchopneumonic lungs and average percentage of affected lung surface. The prevalence of scarring and cranial pleurisy are evaluated based on their presence or absence. Pleurisy is scored and calculated as Actinobacillus pleuropneumoniae Index (APPI) . The CLP was performed at difference slaughterhouses in Thailand during January to December 2019.

Results

Results of Porcine Enzootic Pneumonia lesions (EP-like lesions) and Porcine Pleuropneumonia lesions (App-like lesions) for Thai pigs are presented in Table 1 and Table 2 respectively.

Table 1. EP-like lesions of Thai pigs in 2019.

	P25	Median	P75
% of broncho-pneumonic lungs	53.53%	72.73%	90.00%
Avg. % of affected surface/all lungs	3.78%	6.50%	11.67%
Avg. % of affected surface/pneu-monic lungs	6.23%	10.00%	14.31%
% of cranial pleurisy	0.00%	6.00%	17.91%
% of scar	5.22%	17.65%	36.50%

Table 2. App-like lesions of Thai pigs in 2019

	P25	Median	P75
% of dorsocaudal pleurisy	2.02%	10.20%	30.94%
APP index	0.07	0.31	0.91

Conclusions and Discussion

The results of the lung scoring indicate high prevalence and severity of EP-like lesion. Although the prevalence was similar, the severity of the lesion was higher when compare with the results obtained from all over Asian countries [3]. High percentage of scar lesion indicates that high proportion of Thai pigs were early infected with *Mycoplasma hyopneumoniae*. For App-like lesion, the prevalence was lower but APP index was nearly equal to other countries in Asia [3]. The result from this survey could be benchmark for respiratory health status of pigs in Thailand and useful for swine practitioners to improve their herd health and also improve productivity of the industry.

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Comparison of methods for *Mycoplasma hyopneumoniae* DNA detection in oral fluids

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Introduction

Mycoplasma hyopneumoniae (*MHP*) is an important cause of pneumonia in swine. A challenging aspect of *MHP* prevention is early detection of subclinically-infected animals harboring low levels of the pathogen.
Theoretically, detection of *MHP* DNA by PCR should offer the highest likelihood of detection. *MHP* PCR testing of tracheal samples has been described as the preferred sample for this purpose, but sample collection is labor-intensive, requires trained personnel, and is stressful for the animals. Conversely, pen-based oral fluid samples are easily collected and do not require restraint of pigs. Therefore, the objective of this study was to compare the detection of *MHP* DNA in pen-based oral fluids from animals of known *MHP* infection status. The comparisons involved two extraction procedures and three PCRs.

Materials and Methods

Six-week-old *MHP*-free pigs (n = 39) were randomized to 5 groups: (1) negative control (n = 3 pigs), (2) 1 *MHP*-inoculated pig + 8 susceptibles, (3) 3 *MHP*-inoculated + 6 susceptibles, (4) 6 *MHP*-inoculated + 3 susceptibles, (5) 9 *MHP*-inoculated pigs, i.e., groups differed in the number of inoculated animals. Animals were inoculated intratracheally (10 ml) with lung homogenate containing 1×10^5 CFU/mL (*MHP* 232). All pigs in each group were housed in one pen; each group in a separate room.
Animals were acclimated for 5 days, and followed for 59 days post inoculation (DPI). Pen-based oral fluid samples were collected daily using standard procedures. After collection, samples were vortexed, aliquoted into 10 samples, and stored at -80°C.
Extraction methods evaluated in the study: (1) MagMAX™-96 Pathogen RNA/DNA kit, Applied Biosystems™, Carlsbad, CA; (2) IDEXX RealPCR* DNA/RNA Magnetic Bead Kit, IDEXX Laboratories Inc., Westbrook, ME. DNA extraction was performed on the automated Kingfisher™ Flex System (ThermoFisher Scientific, Waltham, MA).
PCRs evaluated in the study: (1a) TaqMan® Fast Virus 1-Step Master Mix (Life Technologies, Carlsbad, CA) and primer/probe described for Mhp183 (1); (1b) TaqMan® Fast Virus 1-Step Master Mix with the addition of AmpliTaq® 360DNA Polymerase (5U/uL) (ThermoFisher Scientific), and primer/probe described for Mhp183 (1). (2) RealPCR* Master Mix and RealPCR* M hyo DNA Mix, IDEXX Laboratories Inc. PCRs were run to 40 cycles, and performed using the Applied Biosystems® 7500 Real-Time PCR (ThermoFisher Scientific). Testing was performed "blind", i.e., each tube was only identified with a random number. Each procedure (extraction + PCR) was performed using a "virgin" oral fluid sample, i.e., a sample that had not undergone a freeze-thaw cycle. Mixed logistic regression was used to compare the positivity rate among procedures (R program version 3.5.2).

Results

Results are given in Table 1. Although a full factorial design was not used, the data suggested that both extraction method and PCR affected performance. In this study, extraction 1 and PCR 2 provided the highest rate of positivity.

Table 1. *MHP* detection in oral fluid samples.

Procedure		Oral fluid results		
Extraction	PCR	Positive[1]	Negative	Total
1	1a	109[c]	213	322
1	1b	148[a,b]	174	322
1	2	173[a]	149	322
2	2	134[b,c]	188	322

[1]Superscripted letters indicate non-significant (same letter) or significant (different letter) differences in positivity rate ($p \leq 0.05$, Tukey adjustment).

Conclusions and Discussion

Oral fluid testing is widely used for the surveillance of infectious diseases in swine production systems. As has been shown with other pathogens, detection of nucleic acids in oral fluids is highly dependent on the extraction and PCR processes utilized. As shown in this study, these procedures must be optimized to achieve the best possible *MHP* detection.

Acknowledgments

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Conflict of interest

The authors declare no conflicts of interest with the exception that JZ has served as a consultant to IDEXX Laboratories, Inc. on areas of diagnostic medicine independent of this research.

Predictive association between oral fluid and tracheal swab PCR results and clinical signs in *Mycoplasma hyopneumoniae*-inoculated pigs

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Introduction

Mycoplasma hyopneumoniae (*MHP*) is the cause of enzootic pneumonia, a disease that costs the swine industry approximately $400 million annually[1]. Control of *MHP* commonly includes vaccination and antimicrobials. Operative monitoring based on diagnostic testing and syndromic surveillance is needed to decrease the use of antimicrobials in swine. Although detection of *MHP* DNA by PCR from a tracheal swab is the preferred specimen for early detection, oral fluid samples are easily collected and offers prevalence assessment at the herd level. Therefore, the goal of this study was to evaluate the predictive association between oral fluid or tracheal swab PCR results and clinical signs from animals of known *MHP* infection status.

Materials and Methods

Six-week-old *MHP*-free pigs (n = 39) were blocked by litter and randomized to 5 groups housed separately: (1) sham-inoculated, negative control (n = 3), (2) 1 *MHP*-inoculated pig + 8 susceptibles, (3) 3 *MHP*-inoculated + 6 susceptibles, (4) 6 *MHP*-inoculated + 3 susceptibles, and (5) 9 *MHP*-inoculated pigs, i.e., *MHP* prevalence differed by group. *MHP* pigs were inoculated intratracheally (10 ml) with a lung homogenate containing 1×10^5 CFU/mL (*MHP* 232). Tracheal swabs, oral fluids, and individual cough scores (27 min per room) were taken twice weekly by a blinded individual. Water samples were taken once weekly. Air samples were collected three times weekly in Groups 1, 3 and 5. Samples were tested with PCR. Pigs were euthanized 59 days post inoculation (DPI). Mixed linear regression was used to estimate the association between PCR cycle threshold (Ct) values of tracheal samples and individual cough scores, DPI and inoculation status were considered fixed effects, and pig was the random effect. Association between PCR result of oral fluid sample and number of pigs coughing was estimated with mixed logistic regression, DPI was considered fixed effect, and group as random effect. (R program version 3.5.2).

Results

MHP was not detected by PCR in any sample type from the sham-inoculated, negative control group and cough was not observed in this group. A summary of PCR results by sample type, cough score and transmission is presented in Table 1. *MHP* was detected in 90% (18/20) of *MHP*-inoculated pigs at 3 DPI. *MHP* was first detected in oral fluids in group 2 and 5 at 8 DPI and was inconsistently detected in group 2 until 40 DPI but was detected consistently thereafter. *MHP* was continuously detected in oral fluids in group 4 and 5 as earlier as 15 and 12 DPIs until the termination of the study in group 4. *MHP* DNA was detected in 7 air samples and 21 water samples at various time points throughout the study. Coughing was first noted in group 3 at 10 DPI and was later detected in groups 2, 3, and 4 until 59 DPI. A significant inverse association between tracheal sample PCR Ct value and individual cough score [exp(B) = 0.86, P = 0.02] was found. No significant association was found between oral fluid PCR result and number of pigs coughing.

Table 1. *MHP* DNA detection by sample type and cough score in *MHP*-inoculated groups.

	(2) 1 : 8	(3) 3 : 6	(4) 6 : 9	(5) 9
Trachea - Pig-to-pig[1] - 100% detection	DPI 14 DPI 52	DPI 10 DPI 38	DPI 7 DPI 21	- DPI 7
Oral fluid - First detection	DPI 8	DPI 9	DPI 14	DPI 8
Water - First detection	DPI 21	DPI 14	DPI 21	DPI 7
Ind. cough score	97	156	312	301

[1]First pig-to-pig transmission based on trachea PCR positivity.

Conclusions and Discussion

MHP DNA was detected as early as 3 DPI and 8 DPI in tracheal swabs and oral fluid samples, respectively. *MHP* DNA was also detected in air and water samples suggesting these sample types may also assist in the monitoring of *MHP*. A statistically significant inverse association between tracheal sample PCR Ct value and cough score was observed. No such association was found between the number of pigs coughing in a pen and oral fluids. While tracheal swabs remain the gold standard for early detection of *MHP* infection, the data presented in this study suggests that when MHP prevalence is high, oral fluids offers an easier alternative to monitor *MHP* at the herd level.

Acknowledgments

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Comparison of *Mycoplasma hyopneumoniae* exposure methods for gilt acclimation

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Introduction

Mycoplasma hyopneumoniae (MHP) causes chronic respiratory infection and economic losses due to lower growth performance and higher treatment costs. Intentional exposure to MHP during gilt acclimation is intended to provide sufficient time for gilts to develop protective immunity and decrease MHP shedding before entering the breeding herd. Successful application of this approach could reduce the number of positive piglets at weaning, leading to a decreased incidence of MHP in growing pigs. The goal of this study was to compare the efficacy of intratracheal (IT), intranasal (IN) and aerosol (AR) MHP inoculation protocols of live lung homogenate, based on clinical signs, MHP shedding, antibody response, and lung lesions.

Materials and Methods

Seventy-eight of 6-week-old MHP-free gilts were randomized by weight to four inoculation groups: 1) Control (n = 6, Friis medium); 2) IT (n = 24, intratracheal catheter); 3) IN (n = 24, mucosal atomization device); and 4) AR (n = 24, cold fogger mister). The inoculum used consisted of 10 mL lung homogenate with 10^5 CCU/mL MHP strain 232. Aerosol generated with the cold fogger mister were characterized based on amount, size and MHP concentration attached to air particles[1]. Total amount and size of aerosol particles were identified with optical particle counter. MHP concentration estimated with two aerosol collectors (1) Andersen Cascade impactor and (2) impinger. The fogger was tested at three flow rates with the lung homogenate (low, medium and high). Body weight, serum, tracheal and oral fluid samples were collected weekly thereafter euthanasia at 49 day post-exposure (dpe). Trachea and oral fluid samples were tested by PCR, and serum by ELISA. Lung was evaluated for gross lesions and histopathology. Linear mixed regression was used to compare the air particle size, weight gain and antibody response among exposure groups. Differences in MHP shedding among exposed groups was analyzed using area under the curve. Analyses were performed in R program.

Results

The medium flow rate generated a significantly higher amount of air particles, and the aerosol produced was more stable over time. It generated 10^7 MHP DNA copies per m^3 attached to air particles of 3-5µm, which is the size needed for trachea and bronchi colonization. MHP live colonies were attached to all particle sizes measured by the ACI. All pigs from the negative control group remained MHP negative throughout the study. Results from treatment groups are given in Table 1. MHP infection was confirm by PCR in trachea samples at 7dpe in all groups (>87%). No difference in shedding based on trachea samples among exposed routes was observed. Antibody response was detected on average by 21dpe. MHP DNA detection in pen-based oral fluid sample was inconsistent over dpe and among pens.

Table 1. Comparison of MHP exposure methods.

	IT	IN	AR
Shedding (Traquea PCR)			
- *time to 100% gilts positive*	7 dpe	14 dpe	14 dpe
- *Maximum MHP (Cts)*	20.8	21.6	21.03
Antibody response (ELISA)			
- *time to detect fisrt positive*	14 dpe	21 dpe	21 dpe
- *100% detection*	42 dpe	49 dpe	>49 dpe
- *mean S/P ratio*[1]	0.504[a]	0.329[b]	0.281[b]
Weight gain (kg / day)[1,2]	0.58[a]	0.61[a,b]	0.64[b,c]
Lung lesions			
- *Gross (median)*	2.55%	1.73%	1.59%
- *H&E*[3]	17 / 22	19 / 24	19 / 24

[1]Different superscripted letter represent statistical difference ($p<0.05$); [2]Control group: 0.73kg/day[c]; [3]Number of lungs with microscopic lesions per total of gilts.

Conclusions and Discussion

In-depth characterization of the aerosol produced by foggers was previously unknown. This study showed that live MHP is attached to specific airborne particles needed for successful infection. All three exposure routes resulted in MHP infection and caused comparable detection, pathological lesions and similar ADWG. Specific production circumstances may affect the choice of exposure route. For example, IN devices can be utilized to exposed weaned gilts, requiring less pig handling and time compared to the IT route. Aerosol exposure offers the ability to expose large groups of gilts, requires no pig handling, but can be challenging to achieve in large spaces. Serology and tracheal sampling, depending on timing, vaccination and flow limitations will be key to measure level of exposure in acclimated gilts. Practical and consistent gilt exposure methods will result in improved control and elimination programs.

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Diagnostic trends of five swine endemic bacterial pathogens using data from the Iowa State University Veterinary Diagnostic Laboratory (2010-2019)

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Introduction

Streptococcus suis (SS), *Glaesserella parasuis (GPS)*, *Mycoplasma hyorhinis (MHR)*, *Actinobacillus suis (AS)*, and *Mycoplasma hyosynoviae (MHS)* are within the top ten most impactful bacterial pathogens for the swine industry (SHIC, 2020), causing systemic disease, reducing productivity and compromising animal welfare. Accurate diagnosis of disease caused by these agents in the field is challenging due to their commensal ecology, lack of virulence-specific and diagnostic tests, and polymicrobial interactions. The goal of this study was to describe the frequency of disease diagnosis using data from the ISU VDL over a 10-year period.

Materials and Methods

Disease diagnosis was based on pathological assessment in the context of clinical history and results of testing (i.e. bacterial culture and/or PCR). Field cases included in this study were associated with disease diagnosis for the following body systems: cardiovascular, musculoskeletal, nervous, respiratory or systemic from 2010 to 2019. Preferred specimens for etiological diagnosis included: central nervous system tissues, fibrin, heart, joint, kidney, liver, lung, pleura and spleen. Negative binomial regression was used to assess trends for each agent over time. Logistic regression associated agents to respective lesions.

Results

Over a 10-year period, 42,884 porcine cases were identified as having disease in one of the body systems of interest. From those, 8,744 cases received a final disease diagnosis related to at least one of these 5 bacteria. A total of 16% of all cardiovascular cases (2,187), were given either a *SS* or *GPS* diagnosis; 16% of all musculoskeletal cases (1,500) were given either a *SS* or *MHS* diagnosis. For nervous (1,653) and respiratory cases (28,249), 30% and 6% were given *SS* diagnosis, respectively. In systemic cases (15,833), 11%, 10% and 4% were diagnosed with *SS*, *GPS* and *MHR*. Trends in terms of the number of cases diagnosed on a 10 or 5-year period are given in Table 1.

Table 1. Increase (+) or decrease (–) in number of cases with five endemic bacterial diagnoses over a 5- or 10-year period.

Agent	2019 - 2010	2019 - 2015	2014 - 2010
SS	+ 884*	+ 556*	+ 182*
GPS	+ 675*	+ 417*	+ 110*
MHR	+ 301*	+ 184*	+ 72*
AS	+ 164*	+ 119*	+ 42*
MHS	– 8	0	– 5

*Statistical significance ($p < 0.05$)

Overall, disease diagnosis of these 5 agents increased cumulatively 20% per year (Figure 1). Lung (67% of cases) and CNS samples (19%) were frequently used to diagnose *SS*, while lung and fibrin were used to diagnose *GPS* (64%, 35%), *MHR* (62%, 48%), and *AS* (73%, 11%). *MHS* was diagnosed using fluid from affected joints. The primary lesions associated with *SS* disease were serositis (50%), bronchopneumonia (41%), and meningitis (13%). Lesions associated with *GPS* diagnosis were serositis (77%) and bronchopneumonia (28%). Lesions associated with *MHR* diagnosis were serositis (93%), arthritis (8%), and sepsis (9%). For *AS* diagnosis, lesions included bronchopneumonia (57%) and sepsis (45%). *MHS* diagnosis was only associated with arthritis in 133 cases.

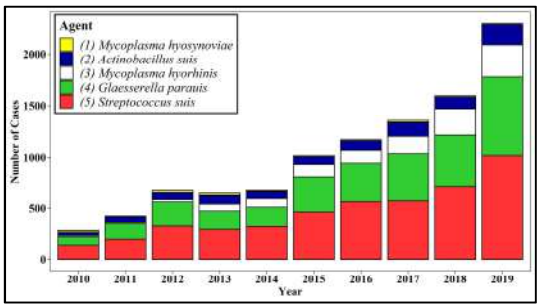

Figure 1. Number of cases submitted to ISU VDL over a 10-year period with disease diagnosis for five endemic bacterial agents.

Conclusions and Discussion

Results demonstrate a marked increase in the annual diagnoses for all agents, with the exception of *MHS*. The factors contributing to increased diagnostic frequency may be varied and include improved diagnostic protocols and tests, changes in diagnosticians, increased awareness and number of submissions, or changes in antimicrobial use. Lungs were frequently submitted for *SS, GPS*, *MHR* diagnosis; however, their use for systemic disease diagnosis requires caution due to the commensal nature of these agents in the respiratory system, compared to systemic sites typically targeted by pathologists (e.g. joint, brain, spleen, etc). The anatomic location selected for sampling coupled with compatible histopathologic lesions and proper animal selection offers a more comprehensive assessment for the contribution of these bacteria to systemic disease. It is critical to refocus the attention on these pathogens, and begin to close important knowledge gaps to develop improved control and prevention strategies.

Influence of using avilamycin, colistin, halquinol and probiotics on performance and occurrence of diarrhea in weaned piglets

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Introduction

Post-weaning diarrhea (PWD) is a disease related to economic losses in weaning piglets (1,3) and the use of antibiotics are usually necessary to avoid or control PWD. However, there is a worldwide trend towards a better use of antimicrobials, encouraging the reduction of critically important antibiotics (8).

Avilamycin is an antibiotic used only in veterinary medicine that acts reducing the amount of *E. coli* fimbriae and consequently reducing the adhesion process in the intestinal lumen (2,5). Moreover, some probiotics can produce antimicrobial compounds that inhibits the production of bacterial toxins or the adhesion of pathogens to the intestinal mucosa (4,7). These two technologies have been shown to be effective in PWD control at field level. For these reason, this study was conducted to compare the performance of piglets fed diets containing critically important antibiotics currently used in Brazilian pig production (colistin and halquinol), compared to an antibiotic for exclusive animal use (avilamycin) and a multistrain probiotic.

Materials and Methods

Three hundred weaned piglets (6.026 ± 0.971 kg) were randomly assigned (30 pens of 5 pigs) to one of six feed treatments under environmental challenge: non-medicated feed during 42 days (T1); avilamycin 80 ppm for 28 days followed by non-medicated feed until 42 days (T2); colistin 200 ppm for 28 days followed by non-medicated feed until 42 days (T3); halquinol 120 ppm for 28 days, followed by non-medicated feed until 42 days (T4); probiotic (200 g/t) composed by *L. plantarum, L. bulgaricus, L. acidophilus, L. rhamnosus, B. bifidum, S. thermophilus* and *E. faecium* for 42 days (T5); and avilamycin 80 ppm for 28 days plus probiotic (200 g/t, same described above) during 42 days (T6). Piglets were individually weighed weekly and at every feed changes. It was analyzed the daily feed intake, weight gain and feed conversion (pen as experimental unit) per week and from the entire experimental period. Diarrhea score was assessed daily according to the following classification: 0 - normal consistency; 1 - semi-solid; 2 - pasty; 3 - aqueous. Parametric data were submitted to ANOVA and averages to Tukey test, and Nonparametric data were assessed using the Chi-square test.

Results

Significant (P <0.001) findings include higher final weight in nursery piglets treated with either avilamycin (20.863 kg), probiotic (21.571 kg), or avilamycin + probiotic (21.363) (considering week factor), compared to colistin (19.689 kg) and no difference to control (20.811 kg) or halquinol group (20.489 kg). In addition, feed conversion on first week was better for avilamycin 80 ppm and avilamycin + probiotic compared to colistin 200 ppm (P <0.001). Avilamycin 80 ppm and probiotic (200g/t) treatments had a higher number of animals over 22 kg at the end of nursery compared to colistin 200 ppm (P <0.001), and without difference (P >0.001), for control, halquinol 120 ppm and avilamycin + probiotic. Diarrhea occurrence in treatments T1, T2, T4 and T6 were significantly reduced compared to T3 and T5 (P <0.001) and the lowest score of diarrhea was found in group T6, followed by group T2, followed by groups T1 , T3, T4 and T5 (P <0.001) (Table 1).

Conclusions and Discussion

Weaned piglets who received avilamycin 80 ppm or avilamycin + probiotic showed better results than animals receiving colistin 200 ppm for: final weight, feed conversion on first week, number of piglets over 22 kg at the end of nursery (T2 only), and occurrence and score of diarrhea. However, there were no differences among the control group, the group with halquinol or the group with probiotic only. Some studies using avilamycin 80 ppm found higher body weight at 28 post-weaning, lower occurrence and score of diarrhea relative to a non-medicated feed (2,5). The results of our study demonstrated that avilamycin, multistrain probiotic or its combination had better results than colistin 200 ppm.

Table 1. Diarrhea occurrence and average score

Treatment	Occurrence*	Score*
Non-medicated	1.3a	0.08a
Avilamycin 80ppm	1.0a	0.07b
Colistin 200ppm	1.5b	0.09c
Halquinol 120ppm	1.2a	0.07c
Probiotic 200g/t	1.5b	0.12d
Avilamycin 80ppm + Probiotic 200g/t	0.7a	0,05a

*Statistical difference on Chi-Square test (P <0.05)

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Mycoplasma hyorhinis isolation and PCR detection rates in polyarthritis and/or polyserositis cases in nursery pigs

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Introduction

Mycoplasma hyorhinis (*M. hyorhinis*) is a commensal bacterium of the pig respiratory tract that can cause fibrinous polyserositis (including pericarditis [PC], pleuritis [PL] and peritonitis [PT]) and polyarthritis in pigs. However, other pathogens such us *Glasserella* (*Haemophilus) parasuis* and *Streptococcus suis* can cause these lesions as well. Confirmation of *M. hyorhinis* implication on these cases requires its detection within the lesions. The present study aimed to assess the *M. hyorhinis* detection rate in nursery pigs showing polyarthritis and/or polyserositis.

Material and Methods

Samples from pigs submitted to the Veterinary Pathology Diagnostic Service at the Veterinary Faculty of *Universitat Autònoma de Barcelona* (Spain) showing fibrinous/fibrous (poly)serositis and/or arthritis were taken.

A swab sample from the observed lesion was taken and immersed in 2 mL of Friis Media. Once vigorously vortexed, this suspension was filtered (0.45 µm). One hundred µL of the resulting filtrate were then mixed with 900 µL of Friis Media and incubated at 37ºC for 1-2 weeks when the media color change (from red to yellow) was evaluated. All *M. hyorhinis* positive cultures were confirmed by the real time quantitative PCR (qPCR)(1). Samples negative to isolation were also tested by direct qPCR.

Results

A total of 23 pigs were included in this study. From these pigs, 4 (17%) had joint inflammation, 9 (39%) suffered from polyserositis and 10 (43%) showed both lesion types. Samples taken from these animals (n=48) included: 15 swabs from swollen joints, 32 swabs from serositis (11 from PC, 9 from PT, 12 from PL) and 1 swab from meninges. These 32 serositis samples were classified as fibrinous (n=23), fibrino-fibrous (n=5) and fibrous (n=4).

Mycoplasma hyorhinis was either isolated or detected in at least one sample in 17 out of 23 (74%) pigs included in this study. Therefore, there were 6 animals with all the samples negative to both techniques.

From the 48 tested samples, *M. hyorhinis* was isolated in 19 (39.6%) samples coming from 12 (52%) animals. Indeed, there were 5 animals with more than one sample positive by isolation. Among these 19 positive samples, 13 (68%, 7 PC, 3 PL and 3 PL) came from serositis and 6 (32%) from articular lesions. Nine out of these 13 serositis samples (70%) were fibrinous, 2 (15%) fibrino-fibrous and 2 (15%) fibrous. From the 6 articular positive samples, 2 were taken from joints (arthritis) and the other 2 from the synovia (tenosynovitis). The qPCR CT values for these 19 samples positive by isolation ranged from 26 to 38 Ct values.

Additionally, 14 (29.1%) samples yielded negative isolation but positive qPCR result. These samples came from 9 animals, 3 of them with up to 3 qPCR positive samples. Among these, 7 were from serositis lesions (50%; 3 PT, 1 PC, 3 PL) and 7 from articular lesions (50%, 4 from arthritis and 3 from tenosynovitis). All but one of these 7 qPCR positive serositis samples were classified as fibrinous. The Ct values for these 14 samples negative to bacterium isolation but positive by qPCR ranged from 30 to 38 Ct values.

Finally, there were 15 (31.3%) samples negative to both techniques (including the meningeal swab). From these 15 samples, 4 came from animals that had other samples positive by one of the used techniques.

Discussion and conclusions

This prospective preliminary study indicated that a high percentage (69%) of samples obtained from polyserositis and polyarthritis cases were positive (either by isolation or by qPCR) to *M. hyorhinis.* Those cases positive by qPCR but negative to isolation (14/48) could be attributable to putative antibiotic treatments or resolving lesions with low amount of existing bacteria.

This result emphasizes the diagnostic importance of this pathogen in cases of polyserositis/polyarthritis.

In order to know if *M. hyorhinis* was the main/unique causative agent of such lesions, presence of the other pathogens able to cause them should be discarded. In any case, *M. hyorrhinis* should be included in the diferential diagnosis of polyarthritis and polyserositis in nursery pigs.

Acknowledgments

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HIPRASUIS® GLASSER reduces mortality of a Glässer's disease outbreak in Colombia

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Introduction

Glaesserrella (Haemophilus) parasuis is a main pathogen affecting pig industry, causing meningitis, polyserositis, polyarthritis and bacterial pneumonia; an infection known as Glässer's disease (1). Antimicrobials have been commonly used to treat this bacterial disease in farm animals, but the emergence of antimicrobial resistance, a serious threat for public health, presses for the implementation of alternatives for disease control (2). Vaccines are the preferred alternatives to control the affection, as they have demonstrated to be useful to prevent this disease (1). The objective of the present study was to determine the effect a commercial vaccine against *Glaesserrella (Haemophilus) parasuis* had on mortality during a disease outbreak.

Materials and Methods

The trial was performed in a 600 sow farrow to finish farm with multiple site production, negative to PRRS, located in Virginia (Risaralda). Weaning averaged 21 days of age. The farm had Glässer disease diagnostic and it was decided to start a vaccination program with HIPRASUIS® GLÄSSER (HIPRA) to control the clinical signs and mortality and compare 3-month period mortality before and after implementing the vaccination program (No vaccine: From Jan 2018 to March 2018; HIPRASUIS® GLÄSSER: From April 2018 to June 2018). Differences between groups were tested through a Wilcoxon test. All statistics were performed with R software.

Results

A significant reduction of mortality was observed in between periods before and after the vaccination started, from 4,1% to 1,6% (P<0.001) (Figure 1a,b).

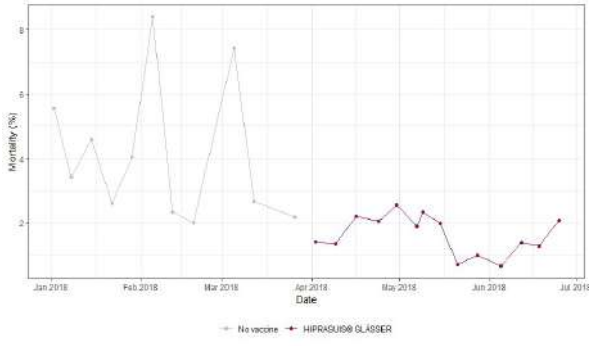

Figure 1a. Weekly mortality distribution during the period.

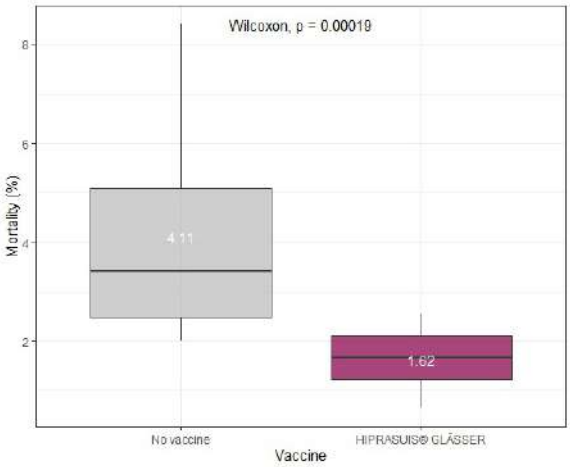

Figure 1b. Mean mortality analysis.

Table 1. Cost of slaughtered animals.

	No vaccine (jan 18-mar 18)	HIPRASUIS® GLÄSSER (apr 18 – jun 18)
Number of animals	1000	1000
Number of pigs to slaugter	958,9	983,8
Mortality (%)	4,11%	1,62%
Vaccination cost	0 COP	2.360 COP
Piglets cost (COP)	207.159.000	207.159.000
Total costs/slaughter animal	216.038 COP	212.969 COP

Although productive parameters and medication cost were not registered, return of investment of the vaccine was calculated with mortality. The 2,5% reduction of mortality achieved by the vaccine treatment represented a reduction of 3.069 COP per slaughtered animal. Hence, 2,3 COP were returned by every 1 COP spent in vaccine.

Conclusions

In this farm, HIPRASUIS® GLÄSSER reduced mortality and reduced the cost of pig production.

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VEROCHECK Diagnostic Kit to monitor Edema Disease in Thai Pig Farming

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Introduction

The main causal agent of Edema Disease (ED) is verotoxin (VT)-producing *E.coli* (VTEC), also known as Shiga-toxin (Stx)-producing *E. coli* (STEC) (1). In affected animals, different clinical forms of the disease have been described ranging from acute to chronic and asymptomatic forms. Verotoxin-2e (VT2e) is encoded by genes present in *E.coli*; however, the percentage of farms with VT2e-positive *E.coli* in Thailand is unknown.

Hence, the purpose of this study was to determine the prevalence of VT2e encoding genes isolated from oral fluid samples from Thai farms using a new diagnostic tool to monitor ED: VEROCHECK.

Materials and Methods

The VEROCHECK method consists of obtaining oral fluid samples from various pens of animals of different ages to detect the orofaecal transmission of VTEC. These oral fluids are eluted using an FTA card and sent to the HIPRA DIAGNOS Laboratory. A real-time polymerase chain reaction (qPCR) test is performed to identify the gene encoding VT2e (2).

For this sampling, a total of 142 oral fluids collected on 24 pig farms from several parts of Thailand was tested during 2019. Five to 7 oral fluids were collected from each farm. From these, 7 farms were identified as suffering *Acute ED* (>6% mortality and submucosal edema), 9 farms with *Chronic ED* (impaired productive performance and a small amount of swelling) and 8 farms with *Subclinical ED* (impaired productive parameters with no clinical signs). Fluid samples were collected from 7 days to 14-week-old pigs.

Results and Discussion

The overall prevalence of VT2e-positive *E.coli* causing Edema disease was detected in 83.3% (20/24) of the farms. Of these, 5 farms with severe acute ED disease were given high antibiotic doses to reduce the spread of illness and pig mortality.

Most positive farms was detected between 4-8 weeks (46/98; 46.9%). This result is probably due to the orofaecal recirculation of *E.coli* when litters are mixed at nursery. On the other hand, the lowest level of positivity was detected in younger animals (1-24d), probably due to the fact that piglets do not yet express the receptors for F-18 *E.coli* (2).

It is interesting to note that *E.coli* was detectedfrom piglets with or without clinical signs of ED. Thus, VEROCHECK diagnostic kit and a real-time PCR diagnostic assay is a good diagnostic standard tool to prove the existence of farms where F18-positive *E.coli* producing Vt2e is endemic, although without clinical signs of Edema disease.

Table 1. VT2e isolated from oral fluid samples of acute, chronic and asymptomatic phases

Clinical signs	Oral fluid collection		
	1-24d*	4-8wk**	9-14wk***
Acute ED	ND****	33/43 (76.7%)	2/3 (66.7%)
Chronic ED	2/14 (14.3%)	8/28 (28.6%)	3/14 (21.4%)
Subclinical ED	ND****	5/27 (18.5%)	5/13 (38.5%)
Total (+VT2e): 58/142 (40.8%)			

*1-3 weeks = Lactation period
**4-8 weeks = Weaning phase
***9-14 weeks = Grower-finisher pigs
****ND = Non-detectable

Oral fluid sampling for Edema disease is emerging as a popular alternative to other tissues because it is a convenient, animal-friendly and cost-effective method (3). Moreover, the proposed methodology is of particular use in production systems with reduced access to veterinary services and farm owners.

This study shows that VEROCHECK methodology can have an impact on the rapid identification of clinical, chronic and subclinical Edema Disease. Monitoring of pig farms is essential to implement corrective measures when needed and enhance the health status of pig herds in Thailand.

Acknowledgements

The authors would like to express their gratitude to farm owners and veterinarians who contributed to the collection of oral fluid samples.

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Clostridioides (Clostridium) difficile in suckling piglets in Italian herds: prevalence and strains characterization

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Introduction

Clostridioides (*Clostridium*) *difficile* is one of the main causes of enteric pathology in newborn piglet (1). Lesions associated with *C. difficile* infection (CDI) involve primarily the large intestine and the clinical signs ranging from watery diarrhea to constipation (2). The aims of the present study were to investigate on prevalence of *C. difficile* in neonatal piglets in Italian herds and to phenotypically and genotypically characterize the isolates

Materials and Methods

The study was carried out between March 2018 and February 2019 and involved 15 breeding herds located in 6 different provinces in northern Italy, a high density area of pig farms. The feces of 30 piglets, belonging to 15 different litters, were sampled from each herd. In all production units samples were collected from both healthy and subjects with diarrhea. For isolation of *C. difficile* was used a three-step method that involve firstly a broth enrichment (TCCFB) followed by alcohol shock and then culture on solid selective medium (TCCFA) (2). *C. difficile* isolated strains were tested for the presence of genes encoding the two major toxins A and B and the binary toxin CDT (3) and typed using the **c**apillary PCR-ribotyping method (4). MICs for vancomycin, moxifloxacin, metronidazole, erytromycin , amoxicilline and tetracycline were evaluated by E-test and analysed according to the EUCAST epidemiological cut-off (ECOFF).

Results

Out of 450 fecal samples, 90 (20.0%) were positive for *C. difficile*. These samples were collected from 13 of the 15 farms enrolled in the survey (86.7%). In these herds the prevalence of *C. difficile* in piglets was between 3.3% and 53.3%. *C. difficile* positive fecal samples from piglets with and without diarrhea are reported in Table 1. Ninety strains were isolated from positive samples and characterized. Genes encoding for toxin A (*tcdA*) and toxin B (*tcdB*) were detected in all the isolates, while genes for CDT (*cdtA/cdtB*) in 96,7%, *C. difficile* strains were classified into five different ribotypes: RT 078 (81.1%), RT 620 (7.8%), RT 126 (6.7%), RT 569 (3.3%) and RT 068 (1.1%). RT 078 and RT 620 were detected in more one herds while RT 569, RT 126 and RT 068 were found each in a single farm. Antibiotic susceptibility was evaluated in 37 *C. difficile* isolates. The MIC50 and MIC90 values obtained are shown in Table 2. All isolates were susceptible to vancomycin and metronidazole. Conversely, 100% of the strains showed a reduced sensibility towards tetracycline and erythromycin. For moxifloxacin only 56,8% of isolated were classified as susceptible .

Table 1. *C. difficile* positive fecal samples from suckling piglets with and without diarrhea in swine herds of north Italy.

Piglets	Total samples No.	Positive samples No. (%)
with diarrhea	179	39 (21.8%)
without diarrhea	271	51 (18.8%)
total	450	90 (20.0%)

Table 2. MIC50 and MIC90 for six antimicrobial agents evaluated in 37 *C. difficile* strains from piglets in Italy

	MIC50 (µg/ml)	MIC90 (µg/ml)
Metronidazole	0,047	0,094
Eritromycin	≥ 256	≥ 256
Tetracycline	3	6
Moxifloxacin	0,75	32
Amoxicilline	0,25	0,38
Vancomycin	1,5	1,5

Discussion and Conclusions

This study showed a significant occurrence of toxigenic *C. difficile* strains in feces of piglets raised in Italian herd. *C. difficile* strains were isolated from both diarrhoeal and asymptomatic subjects. RT 078 was the prevalent ribotype and a significant percentage of strains showed reduced sensibility towards important antimicrobials. Because RT 078 is known to be a relevant cause of CDI in humans (5) this data highlight the necessity of a close surveillance of this infection in swine population, in accordance with a One Health approach to CDI.

Acknowledgments

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Acclimation of Mycoplasma hyopneumoniae-negative replacement gilts naturally exposed to the agent

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Introduction

The etiologic agent of enzootic pneumonia, ***Mycoplasma hyopneumoniae*** *(Mhp)* (8), is responsible for significant damage to animal health and economic losses for global pig production (8). Gilt replacement can pose health risks to the herd when the origin health status is unknown (4). Thus, *Mhp*-free origin herds, considered as high-health status (3), are safer for the destination herd. *Mhp* has slower spread (5) longer shedding period (6), and the introduction of negative gilts into *Mhp*-positive herds increases the risk of transmission from sow to piglets (1). For this reason, with the view to reduce damage to animal health, acclimation of *Mhp*-negative gilts should start as early as possible to allow for the pathogen to be fully cleared by first farrowing (7). This study aims to investigate the dynamics of *Mhp* infection in negative replacement gilts housed in positive commercial farms, and naturally exposed to the agent.

Materials and Methods

Ninety-eight gilts at 150 days of age, from a *Mhp*-negative multiplier, were housed in three positive commercial farms: A, B and C. They were housed in group pens, allowing for contact with sows previously present in the farm, which had been in contact with the resident microbiota. Negative gilts no were vaccinated for *Mhp* and, when necessary, medications were administered individually, and free of any active ingredients that act on *Mhp*. Infection dynamics were assessed with *Mhp* screening by real-time PCR (2) and antibody screening by ELISA (IDEXX®), from individual laryngeal swab and blood samples, respectively. Collections were performed at housing (day 0), at about 15, 30, 60, 90, 120, and 150 days of housing, and in the prepartum period. In farm A, the prepartum period coincided with sampling at 150 days of housing, due to earlier coverages when compared with the age of first coverage in farms B and C.

Results

We found differences in the infection dynamics of the different farms. The first positive *Mhp* detections by PCR occurred in the second week of housing and ranged from 0 to 69% in the different farms. However, at least 30 to 60 days of housing were required for 100% of the gilts in farms B and A, respectively, to become positive. In farm C, it was only after 90 days of housing that 100% of gilts tested positive. At 90 days of housing the number of gilts shedding *Mhp* in farms A and B decreased. In the prepartum period, 57%, 20% and 30% of the gilts still had positive PCR for *Mhp* in farms A, B and C, respectively. These animals can lead to a higher risk of vertical *Mhp* transmission at farrowing (1), with a higher chance of weaning positive piglets (8).

A comparison of PCR and ELISA results showed that the first antibodies were detected in the second week of housing only in farms A and B, and it was only after 60 days of housing that 100% of gilts had positive antibodies in farm A, 90 days of housing in farm B, and 150 days in farm C. These results show that real-time PCR is more sensitive than ELISA for early detection of *Mhp* infections.

Conclusion and discussion

All negative gilts in this study tested positive at least once before their first farrowing. The infection dynamics varied according to the farm, with 57% of gilts still clearing *Mhp* in the prepartum period. In this protocol, where exposure was not controlled, we suggest that gilts be received as early as possible, before 150 days of age, to have enough time to get infected and stop shedding before the first farrowing.

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Evaluation of a recombinant vaccine against *L. intracellularis*

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Introduction

Pig farming represents an important sector for the Brazilian economy, making relevant the development of inputs that reduce the burden of bacterial infections on swine production. Proliferative Enteropathy (PE) is caused by *Lawsonia intracellularis* and is highly prevalent in swine (1). It is characterized by hemorrhagic diarrhea, decreased growth and reproductive rates of animals (2). The available PE vaccines have protection-related limitations, adverse effects and make it difficult to differentiate between diagnoses from vaccinated and infected animals (3, 4). Reverse vaccinology studies have identified a protein conserved in all known strains of *L. intracellularis*, which has 98% similarity of the 16S-rDNA gene with strains that infect other species. This protein is recognized by infected swine serum, suggesting consistent expression by the *L. intracellularis* bacterium and indicates that this protein has the ability to activate the host immune system (5). Therefore, the objective of the present study was evaluate the ability of *L. intracellularis* recombinant protein, fused with TT-Th carrier molecule (rLiTT), to stimulate specific immune response in mice.

Materials and Methods

The LiTT sequence was inserted into the pet/28a vector. The recombinant plasmid LiTT/pet28a was transformed into *E. coli* BL21 Star™ (DE3) cells and the expression induction was performed with 1 mM IPTG for 4 h at 37 °C. Bacteria were recovered by centrifugation, sonicated, and eluted in 8 M urea. Solubilized recombinant proteins were purified using the ÄKTA Primer (GE Healthcare) system. Subsequently the proteins were analyzed using SDS-PAGE. The concentration of the purified protein was determined using the commercial BCA Protein Assay kit (GE Healthcare). To evaluate rLiTT immunogenicity Balb/c female mice were allocated in three groups and inoculated by subcutaneously injection (200 µl) on days 0 and 21: 1. rLiTT (100 µg) adsorbed in 10% aluminum hydroxide [$Al(OH)_3$; Sigma Aldrich)]; 2. Enterisol® Ileitis commercial vaccine (Boehringer Ingelheim) using 1/20th of a recommended dose (2 ml swine dose) and 10% aluminum hydroxide; 3. saline solution plus 10% aluminum hydroxide. Blood samples were collected by submandibular puncture on days 0, 7, 14, 21, 28, 35 and 42 to evaluate the humoral immune response by indirect ELISA. All protocols were approved by the Ethics Committee on Animal Experimentation (CEEA No. 28134-2019) of the Federal University of Pelotas (UFPel).

Results

The rLiTT expression was evaluated by SDS-PAGE and confirmed by Western blot using a monoclonal anti-His antibody which recognized the recombinant protein, showing a band of 18 kDa size. Mice vaccinated with rLiTT/aluminum hydroxide showed antibodies after the prime vaccination (day 14), the level has increased after the boost and keeping high up to 42 days, presenting title 1.6400. The commercial vaccine group also showed humoral response with significant antibodies since the first dose, obtaining 1.3200 title The control group, inoculated only with saline buffer (PBS), did not show humoral response (Figure 1).

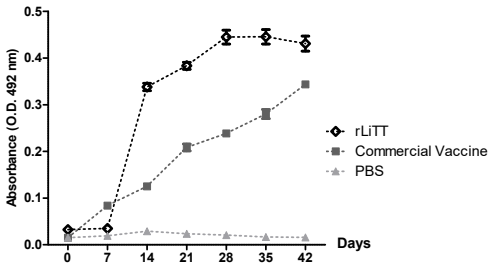

Figure 1. **Evaluation of antibody levels.** Total IgG produced in mice vaccinated with rLiTT and commercial vaccine. Pool of sera were characterized at a single serum dilution (1:100). The mean optical density ($OD_{492\ nm}$) ± standard deviation (bars) from triplicates test is shown.

Conclusions and Discussion

In this study we selected a *L. intracellularis* protein (rLi) fused with TT molecule to be used as antigen to develop an experimental vaccine against PE. The protein rLiTT adsorved in aluminum hydroxide was able to induce humoral imune response in mice, showing IgG dynamics and antibodies title higher than the commercial vaccine. Still, is necessary to characterize the cell response (IL-4, IL-8, IL-17, IFN-y) to determine the potencial of rLiTT to induce protection against PE.

Acknowledgments

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Infection by *Leptospira* spp. in pigs, of backyard farm, slaughtered under municipal inspection in the Midwest of Minas Gerais, Brazil.

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Introduction

Pigs play an important role in the epidemiology of Leptospirosis. Moreover, raising pigs especially backyard production where animals are in close contact is worthening in the one health concept. Leptospirosis occurs worldwide but is most common in temperate or tropical climates. It is an occupational hazard for many people who work outdoors or with animals, such as farmers, slaughterhouse workers and veterinarians, this because of the large spectrum of mammalian reservoirs that harbor and excrete the spirochete. Therefore, the objective of the present study was to determine the infection by *Leptospira* spp. in backyard raised hogs and slaughtered under municipal inspection in the Midwest of Minas Gerais, Brazil.

Materials and Methods

The research comprised a cross-sectional epidemiological study with a quantitative approach, executed in a municipal slaughterhouse, which slaughter backyard pigs. The number of properties and animals used in the project followed a non-probabilistic and convenience sampling, using as a criterion of choice to be registered in the National School Feeding Program and to be slaughtered during the 2018, respectively. Blood, kidney and urine samples were collected from animals. Anti-*Leptospira* agglutinins were evaluated by the microscopic agglutination test (MAT) (1) using a battery of 24 different live antigens including reference samples and native strains isolated in Brazil. The samples that tested positive during the screening tests were analyzed again to define the final agglutination titer. Extraction and PCR were performed in the kidney and urine samples using the protocol according to (2).

Results

From March to December of 2018 eight slaughtering processes were monitored, totaling nine different properties, and 58 animals. Of the 55 serum samples tested at MAT six (10.90%) (Table 1) were reagents for one of the serogroups tested. The Butembo serovar and Autumnalis serogroup were detected in four samples from farms Cb, with titers varying from 800 to 1600, and one sample from property D with title 200. In property G, serovars Guaricura (serogroup Serjoe) and Hardjoprajtino (serogroup Serjoe) were detected with titles of 1600.

Conclusions and Discussion

The serogroups detected in our study are considered not adapted to pigs, therefore, originating from accidental infection (3). The pig infection can be explained by the intimate contact with cattle (4) on the properties studied, due to the form of breeding that is characteristic of the region studied.

Table 1. Farms, number of animals sampled, MAT and PCR results for the diagnosis of *Leptospira* spp. in pigs slaughtered in a abattoir in the Midwest of Minas Gerais, Brazil.

Farms	Total animal	MAT positive	Kidney PCR	Urine PCR
A	07	Neg.	NT	NT
B	08	Neg.	NT	NT
Ca	15	Neg.	Neg.	Neg.
Cb	06	4	Neg.	Neg.
D	08	1	Neg.	Neg.
E	02	Neg.	Neg.	Neg.
F	02	Neg.	Neg.	Neg.
G	08	1	NT	NT
H	02	Neg.	NT	NT
Sample tested		55	33	30
Positive total		6 (10,90)[&]	0	0

[&] Result for tested samples (n=55). NT – not tested Neg. – negative.

Considering the titles interpretation, the animals presented moderate to high level of infection. Since the animals were not vaccinated for the agent, the infection is considered acute. This justifies the non-detection of the agent in kidney and urine by PCR. Despite the low bacterial elimination in cases of accidental infection, pigs can still be sources of infection for humans, who can become infected with the serovars found (5).
Thereby, the pigs raised in backyard farms in the Midwest of Minas Gerais, Brazil are infected with Butembo serovar and Autumnalis serogroup of *Leptospira* spp.

Acknowledgments

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Use of laboratory to evaluate vaccination compliance, vaccine response and disease prevalence

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Introduction

Vaccination programs are designed to potentiate the immune system of the host towards a unique, specific antigen and prevent clinical disease caused by that specific pathogen (Radostitis, 2001). Vaccination programs should be farm specific; based on disease prevalence, pathogen circulation, maternal derived antibodies (MDA), and vaccine response. Vaccines represent an investment and are an important part of preventive medicine. The presence of a pathogen before the vaccine response is achieved, or before vaccination, presence of MDA or poor technique at vaccination could impact protection. Measurable antibodies titers could be a tool to understand vaccine performance. The reduction or lack of antibody typically results in reduced or absence of protection against disease (Hesse, 2008; Niewiesk, 2014). Laboratory use to determine antibody titers could help to plan vaccination programs according to each farm needs. On the other hand, titers might suggest pathogen circulation, vaccine compliance and most importantly will suggest the best time for vaccination once MDA has disappeared (Hesse, 2008).
The objective of this study was to understand the serology response of a new inactivated vaccine for *Lawsonia intracellularis*, to understand pathogen circulation, best vaccination timing as well as vaccine compliance on Canadian farms.

Materials and Methods

Serum and fecal samples were collected from pigs at five Canadian farms using a single 2 mL intramuscular dose at three weeks of age of Porcilis Ileitis® (Merck Animal Health, Madison, NJ, USA). Pigs from control farm were not vaccinated. Approximately 80 pigs per farm were used for this surveillance project. Except the animals from control farm, pigs were sampled at weaning and before vaccination (3 weeks of age), end of the nursery (10 w), mid grower-finisher (14 w), and mid-finisher (17 w). The farms represented a wide range of management: farrow to finish, multi-site production, farrow to feeder pig and raised without antibiotic production system (RWA farm). Samples were collected between April 2016 to November of 2019 and data was used to evaluate vaccine compliance, presence of MDA and pathogen circulation. Farms were classified according *L. intracellularis* challenge level: high challenge (clinical disease presence and positives to polymerase chain reaction, PCR, on fecal samples), and low challenge (no clinic disease and PCR negative). The lab test used was immunofluorescence assay (IFA), an indirect test for antibody detection, with a *L. intracellularis*-specific monoclonal antibody, performed by the University of Montreal.

Results

Serology testing for antibody titer measurement can be used to evaluate the response of Porcilis Ileitis® since the vaccine stimulates antibodies titers. Serology surveillance can be useful to understand the response to vaccination on farms with high pathogen circulation and earlier challenge. In addition serology could be used to determine the best vaccination timing as well as evaluate vaccine compliance. Some farms began vaccinating in the presence of high MDA at weaning, although in these cases was not related to vaccine protection is well known that the best vaccine responses are achieved when MDA have disappeared. (figure 1).

Conclusions and Discussion

Vaccination programs should be farm specific, surveillance will help to understand optimal vaccination timing, understand vaccine compliance and will suggest the best time for vaccination understanding pathogen circulation. Despite all the different situations on each farm, data proved that, one intramuscular single dose of 2ml Porcilis Ileitis® at weaning is efficient to promote a good immune response for animals regardless their field condition.

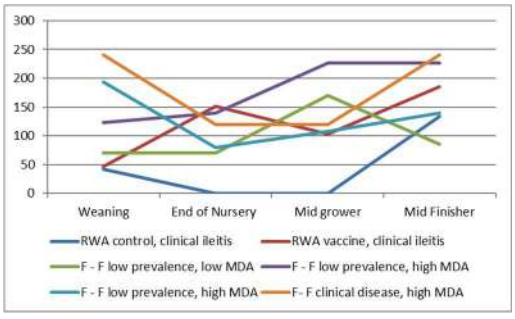

Figure 1. Geo means titration of IFA *L. intracellularis* from five farms in different phases of production.

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A case study of *Mycoplasma hyopneumoniae* like lesions in slaughter pigs

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Background and Objectives

Mycoplasma hyopneumoniae (MH) is a key component of enzootic pneumonia in Vietnam. Swine on Vietnamese farms are often coinfected with other pathogens such as Porcine Reproductive and Respiratory Syndrome Virus (PRRSv), Porcine Circovirus Type 2 (PCV2) and Pseudorabies Virus (PRv). Slaughter pigs from a large-scale single site farrow-finish farm of 1200 sows in Binh Duong province, Vietnam were examined and scored at slaughter for Mycoplasma-like lesions (MLL) from Nov-Dec 2019. In addition, sick piglets were examined at the farm to understand the in-farm MH epidemiology.

Materials and methods

The farm is sited in a rubber plantation and barns are equipped with cooling pads. Sows are mass vaccinated against PRRSv and PRv. Suckling pigs are vaccinated for PRRSv at two weeks of age and vaccinated with PCV2 and MH at three weeks of age. Lungs from 130 slaughter pigs were scored over two nights using the system suggested by Christensen et al (1999). Two trips to the farm were also performed to understand disease conditions of the farm. Four pigs showing clinical signs of respiratory disease were necropsied, two at 6 weeks of age and two at 15 days of age. Gross lesions of lungs and other organs were recorded, and lung samples were microscopically examined. A multiplex PCR was also run to detect MH, PRRSv and PCV2 in lung samples from the four piglets.

Results

Slaughter lung lesions observed are as per Table 1. Both macroscopic and microscopic lesions of lungs from the 4 young pigs suggested absence of MH. PCR results on PRRSV, PCV2 and MH in lung tissues from the four piglets were negative.

In the cases of two suckling piglets and two nursery piglets, both macroscopic and microscopic lesions of lungs from were also indicated that no prominent trend of MLL at young age. PCR results on PRRSV, PCV2 and MH in lung tissues from the four piglets were negative.

Table 1. Scores of MLL and gross findings.

Lesions	Results	Remarks
Percentage of pigs recorded with MLL	102/130 (84.5%)	Consolidation in cranio-ventral lobes was suggestive of MLL
Average score per lung (max 100 points)	5.7	
Interstitial pneumonia	112/130 (86.2%)	Interstitial pneumonia occurred in any lobe. The average score was approximated 2 - 3 (average to severe).
Scars with length >1 cm	36/130 (27.7%)	Presence in any lobes, mainly in cranial and middle lobes
Pleurisy	19/130 (14.6%)	Pleurisy over one or more lung lobes

Discussion

Clinical signs and severity of MH related lesions are dependent on multiple factors, including husbandry and co-infection with other pathogens (Maes et al, 2017). The percentage of pigs exhibiting MLP pigs at slaughter age was high at 84.5% but the average lung score was low - moderate (5.7/100). This suggests that infective pressure on the farm is high, but the current vaccination program is controlling the progress of disease well in the face of high infective pressure. The examination of 15 day old and 6-week-old piglets showed no evidence of MH transmission at young age. MH transmission may be occurring within the herd at the fattener level. The presence of pleurisy and interstitial pneumonia also suggest the presence of other pathogens circulating within the fattening herd

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The economic benefits of vaccination with porcilis ileitis in a commercial farm in Luzon, Philippines

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Introduction

Porcine Proliferative Enteropathy (PPE) caused by the obligate intracellular bacterium *Lawsonia intracellularis* is a common enteric disease worldwide affecting grower pigs between 6 to 20 weeks of age inducing decreased weight gain and feed efficacy, and sometimes death.

Materials and Methods

Weaned piglets of 3 weeks and above were weaned and divided into two Groups. Group 1 (N=156) was vaccinated with Porcilis® Ileitis at 3 weeks of age and maintained on current antibiotic regimen of farm. Group 2 (N=156) was not vaccinated group and maintained on the current antibiotic regimen of farm. Vaccinated and non-vaccinated groups were housed in separate pens from nursery to slaughter to allow for feed consumption measurement and ear-tagged so that no comingling between vaccinated and non-vaccinated groups occurred. Average daily gain, % survivability and final weight gain were computed. On establishing the economic benefit of having an intact control program for Ileitis, current prices and cost assumptions were applied. Data on initial weights and final weights were subjected to statistical analysis using T-test.

Results

Table 1. Production indices of the two treatment groups. (T=Treatment; C=Control).

	Popula-tion	Initial wt. (kg)	Final wt. (kg)	Mort. (%)	Pigs sold	Ave. Age	ADG
T	156	7.75	102.67	21	118	180	0.570
C	156	6.95	91.37	26	116	180	0.508

Final weights between the two groups were significantly different with the treatment group having an advantage of 11.3 kilos per head on the average over the control group. ADG of the treatment group from wean to finish was 100 grams more per day compared to the control group. This may be associated with the integrity of villi intestines which aids the pigs in nutrient absorption that enables optimal growth rates. Subclinical PPE may degrade the integrity of the intestine which may makes the unvaccinated group grow 100 grams less per day. The treatment group has 2 more pigs sold compared to the control. Based on mortality recorded, the control group also had at least 1 mortality directly related to PPE based on necropsy at post mortem.

With the 11.3 kilos advantage earned by the treatment group, the result clearly will cover a vaccination program to control PPE. In this particular study, the treatment group garnered a total of 162,301.50 pesos net benefit over the control that is less the cost of the vaccine.

Conclusion

The study conducted proves that Porcilis® Ileitis can protect pigs from PPE and also reduce economic losses associated. Significant differences in terms of final weights proves that having Porcilis® Ileitis in the health program of the farm can have a beneficial impact on the farm economics. It is possible that this calculation is an under-estimate of the true economic benefit that Porcilis® Ileitis brings, because we were not able to directly measure FCR. Adding in FCR measurements will bring another dimension of economic benefit to the farm, because of improved feed conversion efficiency, which will directly lead to less feed being used to raise pigs.

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Increased average daily gain of pigs vaccinated with Porcilis® Ileitis for the control of *Lawsonia intracellularis*

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Introduction

Lawsonia intracellularis (*Li*) is an obligate intracellular pig bacterium of world importance. With acute (proliferative hemorrhagic enteropathy) and chronic (necrotic enteritis) clinical manifestations, the infection also commonly occurs subclinically in the swine herds, characterized by decreased growth rates, variation in pig size and shedding of *Li* in feces (1). Nowadays, antimicrobials are widely used to control *Li* in Brazilian herds, however, the injectable vaccine against *Li* showed to be more effective in controlling the disease (2) with a 15-fold reduction in bacterial excretion in feces (3). In this context, the objective of this study was to evaluate the impact of herd immunization with the Porcilis® Ileitis injectable vaccine in relation to unvaccinated herds.

Materials and Methods

The field trial was carried out in a Brazilian commercial farrow-to-finish operation, negative for *M. hyopneumoniae* and there is a mortality historical associated from *Li* infection.

Performance was analyzed from 33 unvaccinated lots (Group N_Vac; n = 28,163 pigs) housed between 02/05/2018 to 10/26/2018 and 33 vaccinated lots (Group Vac; n = 27,489 pigs) that received 2 mL of Porcilis® Ileitis vaccine in a single intramuscular dose at 28 days and were housed between 06/11/2018 to 07/18/2019. During the experiment (grower/finisher phase), both animals groups received antibiotics (tiamulin) in feed to control of *Li* according to the prophylactic strategy used on the property. The lots results were considered for the analysis and the variables were: average daily gain (ADG), average initial weight (AIW) and average slaughter weight (ASW); duration of the grower/finisher phase (phase), feed consumption per pig and feed conversion rate (FC). The Proc Mixed Model of SAS™ (2012) was used for analysis of variance. The average initial weight was used as a covariate.

Results

The mean, standard error, descriptive levels of probability of the F test and standard error of the mean (SEM) are described in Table 01. There was no difference between groups for phase, feed consumption per piglet and FC (P>0,05). There was difference between AIW, ASW and ADG. Vaccination of animals with Porcilis® Ileitis increased the ADG by 0.031kg.

Table 1. Mean, standard error, descriptive levels of probability of the F test and standard error of the mean (SEM) for average initial weight (AIW), average slaughter weight (ASW), average daily gain (ADG) and feed conversion rate (FC) of Group N_Vac and Group Vac to Porcilis® Ileitis.

Group	AIW	ASW	ADG	FC
N_VAC	24.3±0,3	116.2±0,7	0.885±0. 004	2.575±0.021
VAC	25.6±0,4	119.3±0,7	0.916±0.005	2.564±0.022
P Value	0.0108	0.0012	<0.0001	0.2126
SEM	0.260	0.521	0.004	0.015

Conclusions and Discussion

Porcilis® Ileitis vaccination improved performance by increased ADG. This is due to the reduction of *Li* lesions and shedding in vaccinated animals which improves intestinal health and increases the nutrient absorption by enterocytes. The bacterium shedding reduction in vaccinated animals was demonstrated previously in a study wich we conducted in this same farm during the same evaluation period (2) (Figure 01). This study demonstrated better efficiency of vaccination with when compared to only antibiotic therapy in feed (2).

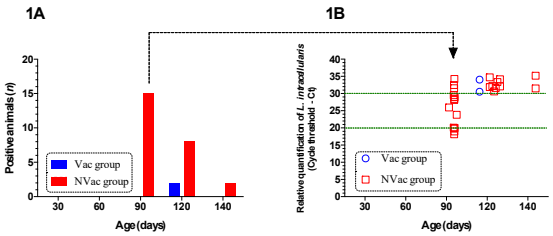

Figure 1A- Positive animals for *L. intracellularis* (15 animal/group/age). **Figure 1B -** qPCR Ct values for *L. intracellularis* detected in feces from pigs vaccinated and unvaccinated (2).

Acknowledgments

Not applicable in these sections.

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Investigation on the use of single or pooled faecal samples and comparison with group saliva samples for *Lawsonia intracellularis* diagnostic in fattening pigs

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Introduction

Lawsonia intracellularis (LI) is very common in pig farms worldwide. Due to the correlation between faecal LI load and pathological findings of proliferative enteropathy[1] the quantification of LI is a valuable tool to estimate the impact of LI as cause of clinical/subclinical problems. Aim of this study was to evaluate whether single faecal samples, pooled faecal samples or group saliva samples can deliver accurate results and which method could therefore be recommended for routine diagnosis in practice.

Materials and Methods

In 15 fattener farms (500-11.000 animals, north west Germany) a total of fifteen faecal samples each (five samples from three different pens) were collected from the floor and one pool was prepared out of the five samples from every pen. Additionally, a chewing rope was offered to the pigs from the same pen to collect saliva. Analyses were performed with the quantitative Lawsonia PCR[2] by Nathues and with the BactoReal Lawsonia kit of Ingenetix. Statistics were done as correlation and inter-rater reliability by Cohen's kappa calculation.

Results

There was a high correlation between results of both qPCRs (BactoReal Ingenetix vs. Nathues[2]; R^2=0.940) in pooled faecal samples (Figure 1).

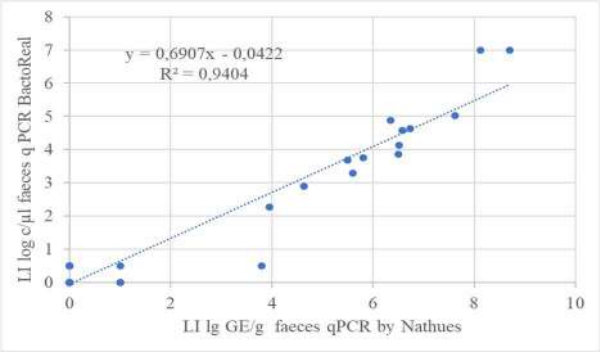

Figure 1. Comparison of faeces aliquots BactoReal LI qPCR Ingenetix (log copies/µl) vs. LI qPCR by Nathues[2] (log GE/g)

The values of single and pooled faecal samples (qPCR LI BactoReal) were also in good agreement (Cohen's kappa 0.71; Table 1).

Table 1. Calculation of Interrater Reliability Cohens Kappa test for LI qPCR BactoReal Ingenetix (log copies/µl) faeces aliquots vs. single samples

Reliability LAW-PCR (Ingenetix) Faeces (Cohen's kappa)		Pool sampling			
		POS	NEG		
Individual sampling	POS	81	13	94	42%
	NEG	19	112	131	58%
		100	125	225	
		44%	56%		

Pr(a)	0,86	86%
Pr(e)	0,51	51%
k	0,71	71%

>0.5 is moderate agreement
>0.7 is good agreement between raters
>0.9 is excellent inter-rater reliability

The correlations between saliva compared to faecal samples (qPCR LI BactoReal) results were lower but still in strong correlation (R^2=0.751; (Figure 2).

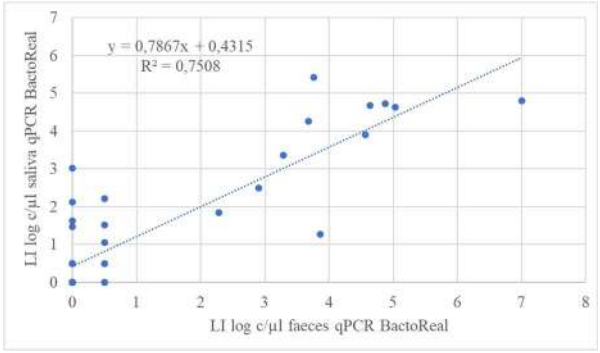

Figure 2. Comparison of LI qPCR BactoReal Ingenetix (log copies/µl) faeces aliquots vs. saliva rope samples

Conclusions and Discussion

For practical reasons the use of pooled samples can be recommended. Due to the close contact between faeces and intestinal mucosa a higher validity can be expected by examination of faecal material. Future studies should be done analyzing LI load of saliva samples by qPCR in defined animals to establish a correlation to the clinical/pathological impact.

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Performance of Porcilis® Ery+Parvo+Lepto vaccine against acute Leptospirosis in the field.

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Background and Objectives

Leptospira is worldwide distributed. There are some serovars affecting pig population, ones adapted (Bratislava, Pomona), others accidentally infecting herds (Icterohaemorrhagiae). Accidental serovars are maintained by other species, like Icterohaemorrhagiae, adapted to rodents. Coexistence in pig farms with rodents is an issue, and sanitary measures are needed to fight against it. Some studies have demonstrated presence of reproductive problems in herds related to the Icterohaemorrhagiae serovar. The aim of this study is to analyze the potential role of sow vaccination against Leptospira in the protection against an acute problem of Leptospirosis caused by Icterohaemorrhagiae.

Material and Methods

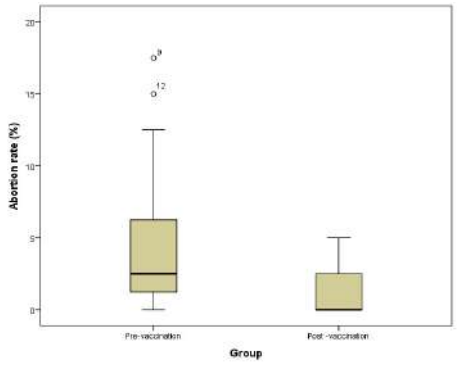
The study was conducted in a 700 sows farm operating a weekly batch system, where reproductive disorders had been reported (abortions, return to estrus, still born). Differential diagnose was done excluding PRRS virus, but *Leptospira* serovar Icterohaemorrhagiae was diagnosed by microagglutination test (MAT, NEIKER, Tecnalia) in serum samples coming from sows with reproductive disorders (paired samples showed seroconversion from negative to titres ≥1/100). Then, two dose program of the vaccine was instated. Two periods were analyzed: prior vaccination (P), 19 weeks before 1st dose; and a vaccinated period (V), 20 weeks after onset of immunity. Reproductive parameters were statistical analyzed by ANOVA (born alive (BA), still born (SB)), and by a non-parametric test KRUSKAL-WALLIS (mummified (Mum), abortion rate (Ab), return to estrus (R), return to estrus in primiparous sows (Rp)). All data were analyzed as an average of the events that occurred in a batch.

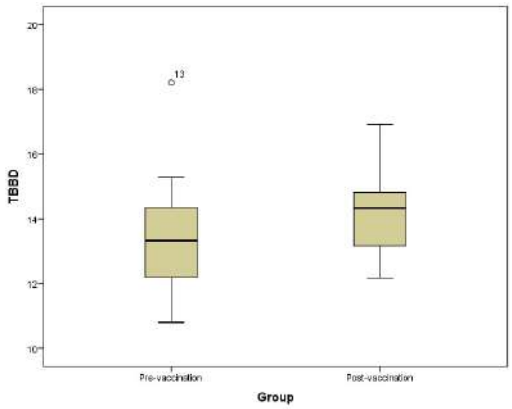
Results

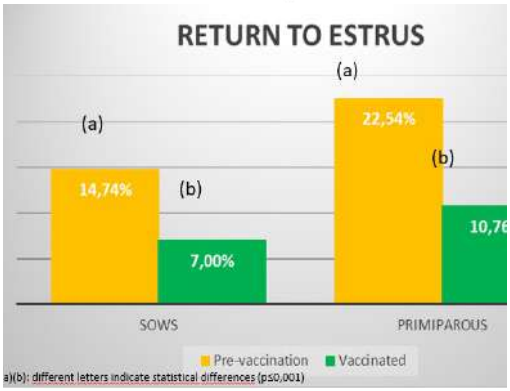
Statistical differences were found in favor of the vaccinated group in the following parameters: Ab: V=1.00% vs. P=4.74% (p=0.011); R: V=7.00% vs. P=14.74% (p=0.001); Rp: V=10.76% vs. P=22.54% (p<0.001); BA: V=12.82 vs. P=11.39 (p<0.001); SB: V=1.21 vs. P=1.86 (p<0.001); Mum.: V=0.071 vs. P=0.155 (p=0.019).

Discussion & Conclusion

Vaccination was a useful tool to control reproductive disorders caused by *Leptospira* Icterohaemorrhagiae, especially in those cases where acute outbreak is affecting reproductive performance. Nevertheless, we must consider, as a limitation of the study, that animals were not bred in the same conditions.







Porcilis® Ery+Parvo+Lepto vaccine against chronic Leptospirosis in the field as an alternative to antibiotic usage

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Introduction

Leptospira is one of the most important worldwide agents causing reproductive disorders. The most prevalent Leptospira serovar in pig population is Bratislava, present at herds in a subclinical way, causing mild reproductive disorders like rise in abortion rate or return to estrus. Besides, reduction in antibiotic usage in EU is cooperating to disease maintenance. The study´s aim was evaluating performance of a multivalent vaccine to control subclinical Bratislava caused leptospirosis, where antibiotics usage was effective to control the disease.

Material & Methods

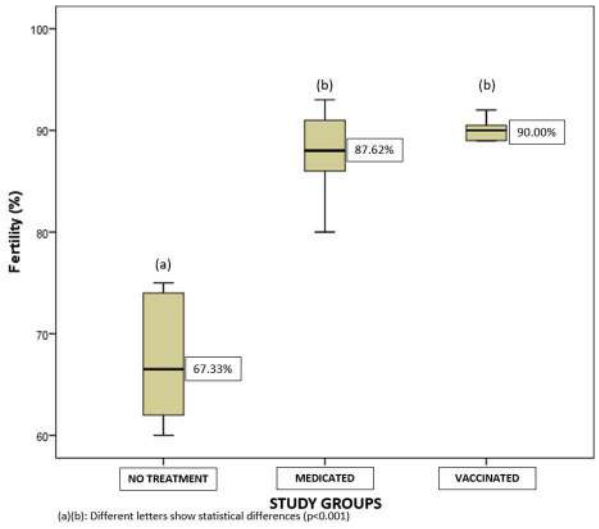
Early abortions (first gestation period) were described in an Iberian genetics farm (1,150 sows). *Leptospira* Bratislava was diagnosed (MAT technique NEIKER Tecnalia, 60% positive samples with titres ≥1/100, from sows with these reproductive disorders). Since then, oxytetracycline (200ppm, water) was administered in the breeding facilities every two weeks for 7 days, thus, one week medicated and one week without medication. Retirement of the treatment derived in gestation failure in the first 5 weeks post-breeding (early abortions). It was then introduced Porcilis® Ery+Parvo+Lepto, 2 doses 4 week separated, and then the antibiotic treatment was ceased. Three periods were determined in this longitudinal study: no antimicrobials (Group: N, 6 months), use of oxytetracycline (Group: M, 26 months) and vaccinated group (Group: V, 8 months). Data were analyzed in a monthly basis, by ANOVA (Fertility %), and by non-parametric test KRUSKAL-WALLIS (early abortions/month).

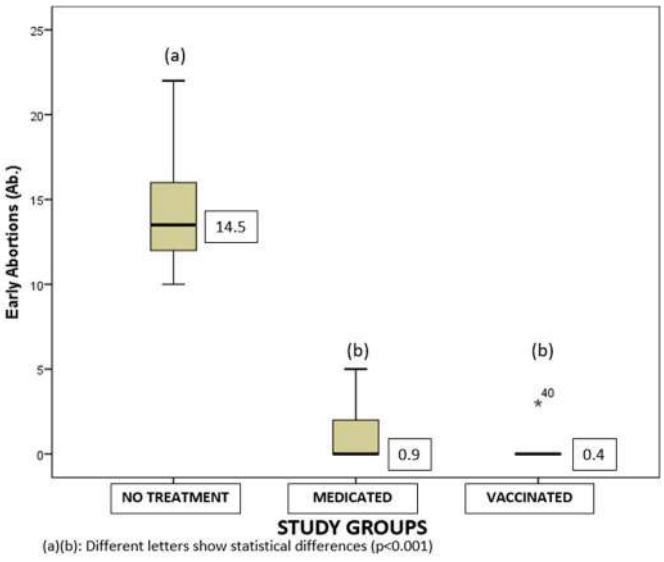
Results

Statistical differences were found ($p<0.001$) in favor of M and V groups, regarding both parameters: fertility N=67.33%, M=87.62%, V=90.00%; and number of early abortions/month N=14.5, M=0.9, V=0.4. Numerical differences were found in favor of V group against M. (Tables Below)

Discussion & Conclusion

In this study the vaccine appeared to be a useful tool to control reproductive disorders caused by *Leptospira* serovar Bratislava, and to reduce the use of antimicrobials. As a limitation, we must consider that animals of the different study groups were bred in different periods of time.





Performance of Porcilis® Ery+Parvo+Lepto vaccine against subclinical Leptospirosis in the field

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Background and Objectives

Reproductive problems are a common issue in the pig production industry. *Leptospira* is one of the most important worldwide agents causing reproductive disorders. The most prevalent *Leptospira* serovar in the pig population is Bratislava, followed by others like Pomona and Icterohaemorrhagiae. Bratislava is present at herds in a subclinical way, causing mild reproductive disorders like rise in abortion rate or return to estrus. Besides, reduction in antibiotic usage in the EU is contributing to disease maintenance. The study´s aim was to evaluate performance of multivalent vaccine Porcilis® Ery+Parvo+Lepto in the control of subclinical leptospirosis.

Material & Methods

The study was conducted in a Spanish farm with 2,900 sows. In the past, the return to estrus rate was too high, especially in gilts. *Lesptospira* serovar Bratislava was diagnosed by the microagglutination test (MAT, NEIKER Tecnalia) in serum samples coming from sows with reproductive disorders (mainly return to estrus and abortions), in some of which were found titres ≥ 1/100. A two doses schedule of the vaccine was tested. Then, two periods were analyzed: prior vaccination (P), 21 weeks before vaccination; and a vaccinated period (V), 21 weeks after the onset of immunity. Reproductive parameters were statistical analyzed by ANOVA (parity rate (Pr), total born (TB), born alive (BA), and still born (SB)), and by a non-parametric test KRUSKAL-WALLIS (mummified, abortion rate (Ab), and return to estrus (R)). All data were analyzed as an average of the events occurred in a weekly batching. Besides, data were analyzed independently considering gilts and multiparous sow.

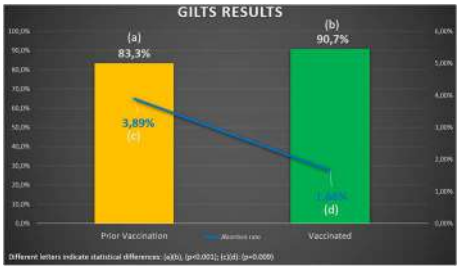
Results

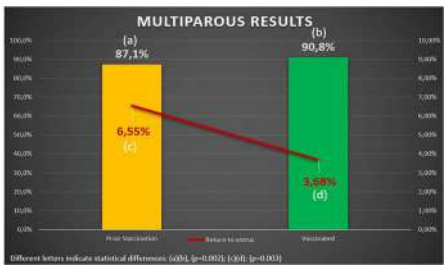
Gilts: statistical differences were found in favor of V group in the following parameters: Pr: V=90.7% vs. P=83.3% (p<0.001); Ab: V=1.66% vs. P=3.89% (p=0.009).

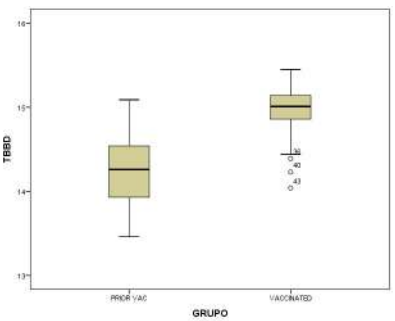
Multiparous: statistical differences were found in favor of V group in the following parameters: Pr: V=90.8% vs. P=87.1% (p=0.002); R: V=3.68% vs. P=6.55% (p=0.003); TB: V=14.94 vs. P=14.23 (p<0.001).

Discussion & Conclusion

This vaccine appeared to be a useful tool to help controlling reproductive disorders caused by Leptospira, even those cases where subclinical infection is subjacent. We must consider as a limitation of study that animals were not bred in the same conditions.







Combination of seroprofile, MIC and PK/PD analysis to manage the APP situation on a hungarian large-scale pig farm
A field study

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Introduction
Actinobacillus pleuropneumoniae (APP) infection has huge economic impact on swine production. Detailed information about the APP strains and the epidemiological situation of the farm are helpful for the successful control of APP while having a prudent use of antibiotics.

Materials and Methods
The mortality rate in mid-fattening on a Hungarian large-scale farm (1.800 sows) increased to 4 - 4.5% with typical clinical and pathological signs of APP. We collected lung samples for laboratory investigation, to identify (serotype) the strains, their antibiotic resistance (ABR), the minimal inhibition concentrations (MIC) and to do pk/pd analysis. Afterwards we collected blood samples of the offspring (starting 1 week of age with 3 weeks intervals, 10 samples/age group). We measured APP APX I-IV toxoids antibodies by ELISA.

Results
The tests identified 2 different serotypes, the "old" (9) and a new (13) one. From the lung samples the laboratory identified 5 APP strains with huge difference of MIC of the two most frequently used AB (amoxicillin 0,25 vs 32 and doxycycline 1 vs 16 µg/ml). We noticed an increase in APX IV in two age groups: the 19 weeks old pigs and in the end of fattening period. The changes of the APX I-III levels were different in these two age-groups which means there could be more unidentified APP serotypes on this farm.

Discussion and Conclusion
Based on the seroprofile results we modified the timing of the vaccination. According to the result of MIC and the PK/PD analysis we selected the effective antibiotics, their concentrations and the way of application (e.g. metaphylactic).
In order to set up an efficient antibiotic control of APP we need more information about the sero-profiles, also in which age groups which serotypes of APP affect the animals. Information about the strain's antibiotic resistance, MIC and the pk/pd analysis proved useful to setup a targeted treatment.

Acknowledgments
MSD AH CER SBU.

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Efficacy of a new vaccine against Erisipelas, Parvovirus and Leptospira (Porcilis® Ery+Parvo+Lepto) to control endemic leptospirosis

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Introduction

The aim of this trial was to evaluate the efficacy of a new vaccine (Porcilis® Ery+Parvo+Lepto, MSD Animal Health) to control an endemic infection of leptospirosis in a commercial sow's farm.

Material and Methods

Tre trial was conducted in a 1200 sow's farm, previously diagnosed as positive of *Leptospira interrogans*, serovar Bratislava. The farm had high percentage of returns in estrus, low farrowing rate, and high percentage of vulvar discharges. To control Leptospira impact, antibiotic treatment was used in a routine basis (oxytetracycline via feed, for 15 days, every 2-3 months), despite reproductive performance was still below objectives. In September 2018, all sows were vaccinated and revaccinated with Porcilis® Ery+Parvo+Lepto, followed by a revaccination scheme every 6 months. Gilts were also vaccinated and revaccinated prior to its introduction in the sow's farm. Antibiotic treatment was stopped 2 weeks after sow's revaccination. To evaluate the efficacy of vaccination, reproductive data were analyzed, comparing data from 6 months prior to vaccination against 5 months after onset of immunity of vaccination (sept and oct 2019 were excluded of the study). Reproductive data were statistically analyzed (one-way ANOVA).

Results

Farrowing rate (FR) was significantly higher in the vaccinated batches (V) than in the not vaccinated ones (NV) (FR: V 90,13% vs 81,96%; p=0,025). No statistical differences were found in fertility until 40 days of gestation, nor in prolificity data (total born, born alive, still births). It was observed a clear reduction in vulvar discharges, that almost disappeared after vaccination. Additionally, none antibiotic treatment was needed after vaccination implementation.

Conclusion

In this study, Porcilis® Ery Parvo Lepto showed to improve the reproductive impact of leptospirosis in an endemic infected farm, as well as to reduce the use of antibiotics destined to its control.

Efficacy of Porcilis® Ery+Parvo+Lepto to control an acute leptospirosis infection

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Introduction

Leptospirosis can have a big impact on reproductive parameters. Its control in Europe has classically been faced via antibiotic treatments or autogenous vaccines. Recently a new vaccine against *Leptospira* was registered in Europe. The aim of this trial was to evaluate the efficacy of this new vaccine to control an acute infection of leptospirosis in a sow's farm.

Material and Methods

The study took place in a 600 sow farm. Between April and June 2018, a reduced fertility rate was detected, reaching values of 78%. Diagnostics proved the presence of an infection with *Leptospira interrogans*, serovar Bratislava. Antibiotic treatment was initiated with Oxytetracycline, and, despite some improvement, fertility was still low. A sow vaccination with Porcilis® Ery+Parvo+Lepto was implemented. All sows were vaccinated in February 2019 and revaccinated 4 weeks later. Re-vaccinations were done per group 10 days post-farrowing. Efficacy of vaccination was evaluated comparing fertility rates from outbreak to vaccination implementation (April 18 to Jan 19) versus the one obtained in the following months after onset of immunity (April to September 2019). The months when basic vaccination was stablished were excluded from the analysis (February and March 2019). Reproductive data were statistically analyzed (one-way ANOVA and Mann Whitney test).

Results

Fertility was significantly higher in the vaccinated batches (V) than in the not vaccinated ones (NV) (V 91,67% vs NV 83,5%; p=0,007). Variability between batches was also reduced (standard deviation V 1,96 vs NV 6,13). Also, a clear reduction of antibiotic use was detected after vaccination. No statistical differences were observed in other parameters such as total born, born alive or still births.

Conclusion

In this study, Porcilis® Ery+Parvo+Lepto showed to be effective to improve fertility and reduce its variability after an acute infection.

Assessment of the humoral immune response against Swine Erysipelas elicited by ERYSENG® PARVO and a trivalent vaccine

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Introduction

Prevention of Swine Erysipelas (SE) is best accomplished by immunization programmes. Both humoral and cell-mediated immunity play a role in the host defense against SE infection. The presence of cellular-mediated immunity against SE was confirmed in mice experimentally immunized with acapsular *E. rhusiopathiae*[1]; however; its relative contribution to protection and the bacterial antigens involved are unknown at the present time. This study therefore targeted the humoral immune response that could be quantified by different commercial indirect ELISAs[2].The aim of this study was to evaluate and compare the humoral immune response against SE elicited by ERYSENG® PARVO and a trivalent vaccine over a period of 71 days.

Materials and Methods

A controlled and blinded experimental trial was performed in SE serologically negative animals. 30 animals were randomly assigned to 3 different groups (n=10). Group 1 (G1) was vaccinated with ERYSENG® PARVO (bivalent vaccine against SE and Porcine Parvovirus (PPV) with an adjuvant based on ginsenosides), group 2 (G2) with Vaccine B (trivalent vaccine against SE, PPV and *Leptospira interrogans sp.* adjuvanted with α-tocopheryl acetate), and Group 3 (G3) was injected with PBS as the control group. All the groups were vaccinated and revaccinated intramuscularly following the summary of product characteristics (SPC) of each product.

Serum samples were taken on days -21, 21, 36, 49 and 71 after vaccination, day 0 being the day of the first dose. SE serology was performed using a commercial indirect ELISA kit without bias versus any of the vaccines used[2].

Results

At the beginning of the study, all the animals were negative against SE, and the control group remained negative for the whole duration of the trial.

On the other hand, after the basic vaccination scheme, SE-antibody titres in G1 were the highest throughout the study, being statistically different from G2 (Mann-Whitney U test; $p<0.05$) from day 36 to day 71 of the study.

G1 and G2 remained above the cut-off (≥40 IRPC) within the study, with the maximum antibody levels occurring on day 49 of the study. However, G1 reached mean SE-antibody levels of more than 103 IRPC on days 36, 49 and 71 after vaccination, whilst G2 reached a maximum of 92.3 only on day 49 of the study (Figure 1).

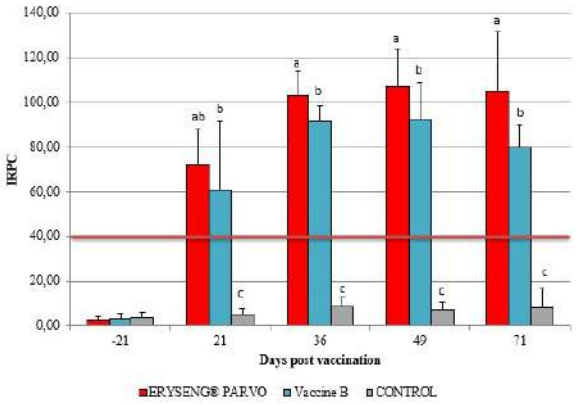

Figure 1. Results are represented as average and standard deviation. Different letters indicate statistically significant differences (Mann-Whitney U test; $p<0.05$). The slashed red line indicates the cut-off (40 IRPC).

Conclusions and Discussion

A whole vaccination cycle for a gilt will involve a period of over 166 days, as the basic vaccination scheme starts 42 days before artificial insemination, followed by 114-115 days of gestation. The next vaccination should be given on day 10 of lactation so that a strong and long-lasting humoral immune response will be needed against SE.

The humoral immune response elicited by both vaccines was different in terms of dynamics and intensities, with the response produced by ERYSENG® PARVO being notably higher compared to vaccine B with statistical differences on days 36, 49 and 71 after vaccination., this could lead to have a better herd immunity against SE.

Acknowledgments

The authors wish to thank the UCAM unit of HIPRA for their technical support.

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Assessment of the safety of ERYSENG® PARVO compared to a trivalent vaccine

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Introduction

The maximum level of antibodies against Swine Erysipelas (SE) and Porcine Parvovirus (PPV) is reached 21 days after vaccination with the last dose[1], so that in sows, the optimum time for vaccination is during lactation at 21 days before the next artificial insemination.

Recently, different studies have shown how important the safety of different vaccines is when they are administered during lactation, as they could affect feed intake, milk production, and consequently, piglet performance during lactation[2].

The aim of this study was to assess and compare the safety, in terms of local reactions and increase in body temperature, of two different reproductive vaccines.

Materials and Methods

A blinded and controlled experimental trial was performed with 27 sows during lactation. These animals were randomly assigned to three groups; Group 1 (G1, n=10) was vaccinated with ERYSENG® PARVO (bivalent vaccine against SE and PPV adjuvanted with HIPRAMUNE® G), Group 2 (G2, n=10) was given Vaccine B (trivalent vaccine against SE, PPV and *Leptospira interrogans sp.* adjuvanted with α-tocopherol-acetate), and Group 3 (G3) was injected with PBS as a control group. All the groups were vaccinated on approximately day 16 of lactation following the manufacturer's instructions.

Rectal temperature (RT) and local reactions (LR), in terms of inflammation at the inoculation point, were evaluated 24 hours before vaccination, at the time of vaccination (0 hours), 6 hours post vaccination (hpv), 24 hpv and 48 hpv.

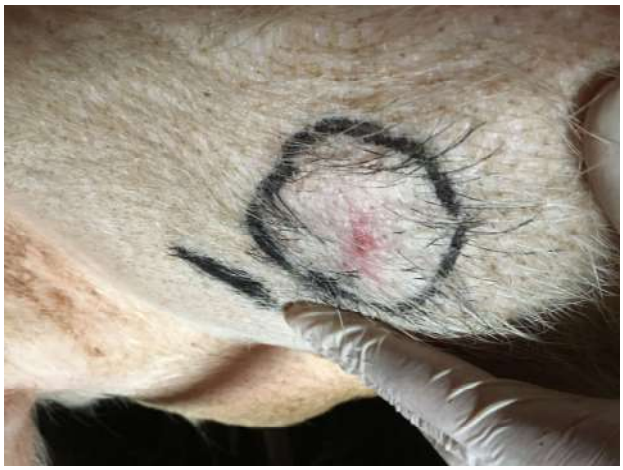
Results

At the time of vaccination, none of the animals had fever. At 6 hpv, significant differences were found between G2 and the control group (p<0.05), with more than half a degree difference. Furthermore, the increase in rectal temperature in G2 was higher than in the group vaccinated with ERYSENG® PARVO just after vaccination and until the end of the study, as seen in Table 1.

Table 1.Mean increase in RT of the different groups during the study. An ANOVA test was performed to analyse the differences between groups. Different subscripts mean significant differences (p<0.05).

Time	Control	Vaccine A	ERYSENG® PARVO	P-value
Reference	-1 day	-1 day	-1 day	
0	0.345	0.325	0.336	0.99
6 h	0.288^{A}	0.865^{B}	0.699^{AB}	0.03*
1 day	0.168	0.517	0.293	0.35
2 days	0.33	0.07	0.016	0.82

There were also significant differences in terms of LR (inflammation at the inoculation point) between G2 (mean of 0.4 cm diameter) and G3 (mean of 0 cm diameter) at 6 hpv (p<0.05). No significant differences were observed between G1 (0.2 cm diameter) and the control group at any time point in the study.



Picture 1. Assessment of LR (Inflammation at the inoculation point) of a sow from Group 2.

Conclusions and Discussion

The present study was intended to explore differences in safety, and the results indicate a difference in terms of an increase in body temperature and local reactions between G2 and the control group. Thus, these differences could have a negative impact on sow performance during lactation (low feed intake and reduced milk production) and consequently, a worse outcome for piglets at weaning. Further studies should be performed to determine how the lack of safety of some reproductive vaccines could affect reproductive performance in sows during subsequent insemination and gestation periods.

Acknowledgments

HIPRASTAT team for their statistical support.

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Evaluation of turbinate atrophy in slaughtered pigs in Thailand by nasal lesion scoring

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Introduction

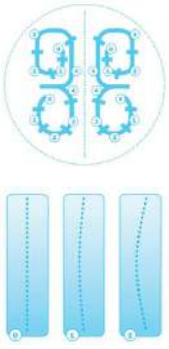
Porcine atrophic rhinitis (AR) caused by toxigenic strains of *Bordetella bronchiseptica* (Bb) and *Pasteurella multocida* type D (PMT) is a disease with a seriously underestimated economic cost. This disease has two forms of presentation; non-progressive AR (NPAR), produced by Bb, induces growth retardation and poor health status (sneezing) and progressive AR (PAR), induced by toxigenic *P. multocida,* causes severe cases of nasal septal deviation (1). Other pathogens historically associated with AR are *Haemophilus parasuis*, *Mycoplasma hyorhinis*, *Streptococcus suis* and viral infections such as swine influenza. In Thailand, disease knowledge is poor and how the swine population is affected is unknown. The main objective of this study was to evaluate the prevalence of nasal lesions associated with AR in Thai slaughterhouses.

Materials and Methods

A farrow-to-finish unit with >800 sows was selected for this study during 2019. Three hundred eight pigs from 15 farms from northern (N), northeastern (NE), eastern (E)and western (W) regions of Thailand were evaluated. A blind analysis was carried out for turbinate bone atrophy and nasal septum deviation in accordance with the scoring shown in table 1 (2). Pigs from both vaccinated and non-vaccinated sows were included in the study.

Table 1. Nasal lesion scoring system.

Lesion grade
Turbinate atrophy (16 points)
0 – Normal
1 – < Half absence
2 – > Half absence
3 – Straight turbinate
4 – Complete absence
Septal deviation (2 points)
0 – Normal
1 – Slight deviation
2 – Deviated septum
Total score (18 points)



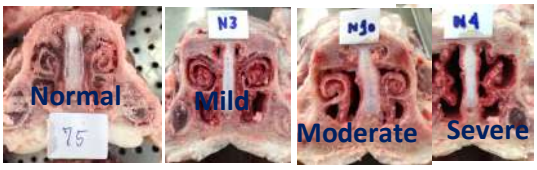
Lesions in this study were divided into 4 grades: score 0 = no lesion (Normal), score 1-4 = mild lesions, score 5-11 = moderate lesions; 12-18 = severe lesions

Results

181 out of 242 fattening pigs' noses (74.8%) derived from unvaccinated sows showed lesions. 57 out of 66 fattening pigs' noses (86.4%) derived from vaccinated sows showed affected pigs. Mild nasal lesions were mostly observed in fattening pigs from both unvaccinated sows and vaccinated sows.

Table 1. Nasal sections of pig snout.

Nº. farm	Region	Nº. sample	Nasal score (%)			
			0	1-4	5-11	12-18
Fattening pigs from unvaccinated sows						
1	N	20	15	40	40	5
2	W	50	18	70	12	0
3		33	57.6	42.4	0	0
4	NE	20	20	75	5	0
5		20	5	60	30	5
6		20	20	55	25	0
7		15	20	53.3	26.7	0
8		20	50	35	15	0
9	E	20	15	70	15	0
10		40	10	62.5	27.5	0
11		5	20	80	0	0
Total		**242**	**25.2**	**54.5**	**19.4**	**0.8**
Fattening pigs from vaccinated sows (No RHINISENG® vaccine)						
12	N	20	15	45	40	0
13	NE	10	10	70	20	0
14		16	12.5	50	37.5	0
15	E	20	15	60	25	0
Total		**66**	**13.6**	**54.5**	**31.8**	**0**



Conclusions and Discussion

This study shows the presence of PAR on Thai swine farms all over Thailand. Moreover, NPAR is also present in pigs vaccinated against AR. The nasal lesion scoring system used in this study was helpful for evaluation of farm status in terms of PAR.

Therefore new AR vaccines should be tested and evaluated on Thai farms in order to improve the current status of PAR and NPAR in Thailand.

Acknowledgments

With grateful acknowledgment to the many swine farm owners in Thailand for providing the samples and for their encouragement.

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Prevalence of Swine Atrophic Rhinitis in Pig Farms in Peninsular Malaysia

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Introduction

Swine atrophic rhinitis (AR) is an important disease in pig production as it affects growth performance (1) and leads to economic losses. It is caused by the toxigenic strains of *Bordetella bronchiseptica* (Bb) and *Pasteurella multocida* type D (PMT), which result in the characteristics turbinate atrophy and septum deformation in nursery and fattening pigs. AR has been documented in many countries, but limited studies were conducted in Malaysia. The objective of this study was to survey the AR situation in Malaysia pig farms via oral fluid (OF) and nasal lesion scoring (NLS) system.

Materials and methods

A total of 11 pig farms located in Peninsular Malaysia were selected for sampling between July and December 2019. The OF samples were collected from gilts, nursery pigs (4-11 weeks old) and fattening pigs (12 weeks old – slaughter) in each farm and inoculated in FTA elute cards before sending to DIAGNOS® Laboratory (HIPRA) for real-time polymerase chain reaction (qPCR) detection of Bb and PMT genes. Furthermore, a total of 183 fattening pigs from these farms were sampled for NLS by sectioning for the snouts following the European Pharmacopoeia guidelines (2). Each of the four scrolls of the ventral turbinate bones and nasal septum deviation were scored on the scale of 0-4 and 0-2, respectively, giving rise to the maximum score of 18.

Results

The Bb DNA was detected in all farms with 100% positive in the fattening pigs. On the other hand, the PMT DNA was found in 27% of the farms (Table 1).

Table 1. Prevalence* of Bb and PMT genes in 11 pig farms in Peninsular Malaysia.

Farm No.	Bb			PMT		
	Gilt	Nursery	Fattening	Gilt	Nursery	Fattening
1	+	++	+			
2	+	++	+			
3			++			
4	+		++			+
5	+	+++	++		++	
6	++		++			
8			+			
9		++	+			
10	+	+	+			
11	+	++	++		+	

*Positive bacterial DNA was quantified as lower (+), moderate (++), or higher (+++) amount.

From the NLS analysis, the average score was 8.7, indicating a moderate lesion. The highest NLS score achieved was 18, which represent a severe lesion. The lowest score seen was at a healthy level of 3.1. Nasal septum deviation was present in 3.5% of the samples (Figure 1).

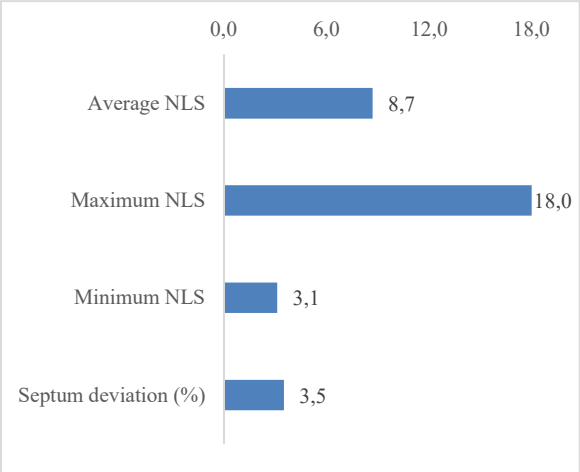

Figure 1. Summary of NLS on 183 fattening pigs from 11 farms.

Discussion and conclusions

Results from the qPCR on the OF samples and NLS analysis showed that AR is present in commercial pig farms in Peninsular Malaysia. The prevalence of Bb was the highest among fattening pigs as compared to gilts or nursery piglets. While the presence of PMT was only seen in 3 farms with a moderate level average NLS score. The potential impact of this disease on the farm production performance should not be overlooked. Prevention by vaccination with vaccine antigen containing attenuated Bb and PMT toxoids is recommended for AR control in the pig farms (3).

Acknowledgments

We would like to thank all farm owners in Malaysia who had contributed and been involved in this survey, as well as Dr Fong Chee Wee for his invaluable technical contributions to this study.

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Occurrence of *Bordetella bronchiseptica* and *Pasteurella multocida* associated with non-progressive atrophic rhinitis on pig farms in Thailand via oral fluids

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Introduction

Porcine atrophic rhinitis (AR) is a chronic respiratory disease caused by *Bordetella bronchiseptica* (Bb) and toxigenic *Pasteurella multocida* (PMT) (1). Currently, AR is divided into two forms: so-called non-progressive AR (NPAR), produced by Bb, which affects growth performance and predisposes to other bacterial infections and progressive AR (PAR), which is produced by both Bb and PMT and induces severe cases of nasal septal deviation (2). Oral fluid (OF) sampling for AR is emerging as a popular alternative to tissues because it is convenient, is an animal welfare-friendly method and is cost-effective (3). In Thailand, the presence of Bb and PMT on farms remains unclear, but poor productivity parameters could indicate the presence of these pathogens as an asymptomatic form of AR. The aim of this study was to check the presence of Bb and PMT in different areas in Thailand.

Materials and Methods

A total of 10 swine farms with different farm managements and herd sizes (medium- and large-scale) from the Chaing mai (N), Chumphon (S), Chonburi (E), Kanchanaburi (W), Ratchaburi (W) and Lopburi (C) provinces of Thailand was evaluated. All the farms were suffering from porcine respiratory disease complex (PRDC). Pigs from 4 out of 10 farms had never been immunized by AR vaccine (NV). OF samples from different pens and different ages (gilts, sows and pigs of 4-27 weeks of age) were eluted using FTA cards and sent to the DIAGNOS® Laboratory (HIPRA Thailand). DNA extraction and a real-time polymerase chain reaction (rt-PCR) test (3, 4) was performed for both Bb and PMT detection.

Results

The overall prevalence of Bb on 10 swine farms was demonstrated by a real-time PCR method showing 100% positivity (10/10), whereas PMT was observed on only 1 pig farm (10%). Investigation of Bb and PMT in different stages of pigs from non-vaccinated and vaccinated breeder pig farms is shown in Table 1. The highest Bb positivity was observed in fattening pigs from NV farms.

Conclusions and Discussion

It is accepted that *B. bronchiseptica* and toxigenic *P. multocida* may have been circulating in this region as endemic bacteria, whether or not AR vaccine is used. However, the outcome of low amounts of DNA in oral fluid samples is not directly linked to the clinical stage of AR and its virulence. Moreover, our findings provide useful data to facilitate a better understanding of the status of these pathogens in Thailand. Control measures for PAR and NPAR will be relevant to future work on testing the field efficacy of AR vaccines in Thai pig farming.

Acknowledgments

We gratefully acknowledge farm owners for providing OF samples and Dr. Sithipon Jongpattanasombut (BM, HIPRA Thailand) for his excellent support.

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Table 1. Detection of Bb and toxigenic PMT in pigs from vaccinated and non-vaccinated breeder pigs in Thailand, 2017-2019.

Group	Type of OF	Nº. OF sample	Bb pos	Bb positive (pos)			PMT pos	PMT positive (pos)		
				+	++	+++		+	++	+++
Gilts	NV	9	5/9	4/5	1/5	0/5	0/9	0/0	0/0	0/0
	V	30	12/30	9/12	2/12	1/12	1/30	0/1	0/1	1/1
Sows P1-P2	NV	-	-	-	-	-	-	-	-	-
	V	6	2/6	1/2	1/2	0/2	0/6	0/0	0/0	0/0
Grower-finisher pigs	NV	14	5/14	5/5	0/5	0/5	0/14	0/0	0/0	0/0
	V	9	2/9	2/2	0/2	0/2	0/9	0/0	0/0	0/0
Fattening pigs	NV	41	25/41	17/25	8/25	0/25	0/41	0/0	0/0	0/0
	V	16	11/16	11/11	0/11	0/11	0/16	0/0	0/0	0/0
Pigs at Slaughterhouse	NV	4	1/4	1/1	0/1	0/1	0/4	0/0	0/0	0/0
	V	2	0/2	0/0	0/0	0/0	0/2	0/0	0/0	0/0

* Bacterial DNA was detected in low (+), medium (++) and high (+++) amounts.

Detection of *Mycoplasma hyopneumoniae* in processing fluids in the event of a clinical respiratory disease outbreak

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Introduction

Diagnosis of early infection with *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) in swine breeding herds remains challenging. Recently, *M. hyopneumoniae* has been detected in processing fluids (PF), which consist of serosanguineous exudates from tissues obtained after tail docking and castration of newborn piglets (1). This study investigated the putative use of PF to detect *M. hyopneumoniae* in the event of a clinical respiratory disease outbreak in a previously *M. hyopneumoniae* negative sow farm.

Materials and Methods

The study was performed in a 5,450 sow-breeding farm deemed as negative for *M. hyopneumoniae* and clinically stable for porcine reproductive and respiratory syndrome virus (PRRSV). To monitor for PRRSV, the farm routinely tested three pooled PF of 15 litters each on a weekly basis. Additionally, forty-five individual litter samples were also stored once monthly and tested if needed.

The first week of August 2018, an outbreak of respiratory disease was detected, and diagnostic laboratory tests confirmed the coexistence of *M. hyopneumoniae* along with other bacteria and porcine circovirus type 2.

Once the porcine respiratory disease complex outbreak was confirmed, a retrospective testing of PF for *M. hyopneumoniae* by real-time PCR (2) was performed. Thus, 90 PF collected and stored between March 19 and October 8, 2018, were tested. In addition, VetMAX™ *M. hyopneumoniae* control synthetic DNA (Life Technologies) at a concentration of 1,000 copies μL^{-1} was used for relative quantification in suspect or positive PF samples.

Results

All pooled PF tested negative for *M. hyopneumoniae* by real-time PCR, except 3 suspect samples collected on August 13, 20 and 27, which showed Ct values >37. The three suspect PF were collected while the clinical respiratory disease outbreak was taking place. The positive PF from August 27 was composed of PF from 12 litters that were individually tested by real-time PCR. All but one litter PF with a Ct value of 32.21 were real-time PCR negative. The DNA of these PF was relatively quantified by a standard curve method. The number of DNA copies per mL of PF were 2.2, 1.4 and 3.5 x 10^3 for the samples from August 13, 20 and 27, respectively. The individual litter PF had 350 x 10^3 copies mL^{-1} (Figure 1).

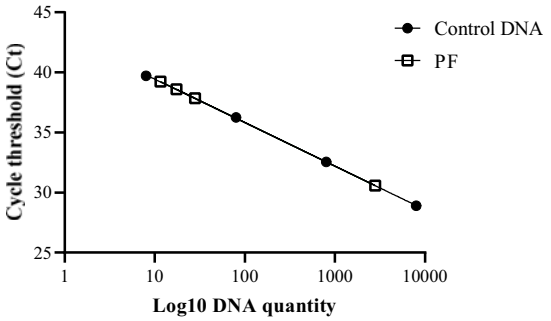

Figure 1. Real-time PCR standard curve graphically represented as a semi-log regression line plot of Ct value vs. log of input DNA. Blue dots represent the ten-fold serial dilutions of the control DNA whereas the green squares represent the retested PF samples.

Conclusions and Discussion

Processing fluids have been postulated as an appropriate sample for monitoring PRRSV in swine herds (3,4) and recently published data also showed detection of *M. hyopneumoniae* in this sample type (1). This latter finding is difficult to explain as *M. hyopneumoniae* is regarded as an extracellular pathogen that resides uniquely in the respiratory tract of pigs (5). While environmental contamination cannot be ruled out, the role that this sample could play in detection of early stages of *M. hyopneumoniae* infection deserves to be further evaluated.

Our results provide new insights into the value that testing PF may have to detect *M. hyopneumoniae* in breeding herds.

Acknowledgments

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Lung homogenate optimization for successful *Mycoplasma hyopneumoniae* exposure in gilts during acclimation

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Introduction

Effective gilt acclimation protocols to *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) pursue to reduce bacterial shedding at first farrowing and, consequently, decrease pre-weaning prevalence and respiratory problems in later stages of production (1). One method for gilt acclimation to *M. hyopneumoniae* entails the use of herd-specific lung homogenate for intentional exposure (2). However, characterization of lung homogenate has been minimally performed, which poses risks for exposure failure.

Lung lesions associated to *M. hyopneumoniae* consist of consolidated areas typically affecting the apical and middle lobes and, eventually, cranial part of diaphragmatic lobes (3). Whether these areas are more suitable to produce lung homogenate to expose gilts to *M. hyopneumoniae* is not known. The aim of this study was to determine the optimal conditions for preparation of lung homogenate by evaluating the bacterial load in different anatomical lung sections by means of real-time PCR.

Materials and Methods

A graphical representation of the study experimental design is shown in Figure 1. A total of nine lung donor pigs were selected from three different farms and production systems (n=3/farm). Donor pigs were selected based on cycle threshold (Ct) values from deep tracheal catheters (DTC) tested for *M. hyopneumoniae* by real-time PCR (4). Pigs were categorized into the following Ct value groups: low (Ct ≤24), medium (Ct 25-30) and high (Ct 31-39). Selected pigs were humanely euthanized, and their lungs collected. Lung lesions were scored and bronchial swabs from each lung lobe obtained. Each lobe was dissected, and tissue was blended at a 70:30 proportion of tissue and Friis medium using a Ninja® Blender (©2019 SharkNinja Operating LLC). Real-time PCR for *M. hyopneumoniae* detection was performed on bronchial swabs and lung lobe-specific homogenates (5 replicates each).

Statistical analysis was performed using ANOVA to compare Ct values of lung homogenates from different lung lobes. In addition, the correlation between Ct values from bronchial swabs, lung lobe-specific homogenates and lung lobe lesion scores were evaluated using Pearson coefficient.

Results

Lesions were observed on all lungs. All lobe-specific bronchial swabs and 85% of lung homogenates were positive for *M. hyopneumoniae*, regardless of the presence of lung lesions. All lung lobe homogenates were positive from pigs with low DTC Ct values, in contrast to pigs with medium and high DTC Ct values, where there were both negative and positive lung lobe homogenates. Mean Ct values in lung lobe homogenates were significantly lower in low DTC Ct pigs compared to medium and high DTC Ct pigs ($p<0.0001$), along with less variation of lung homogenate Ct values across all lobes for low DTC Ct pigs.

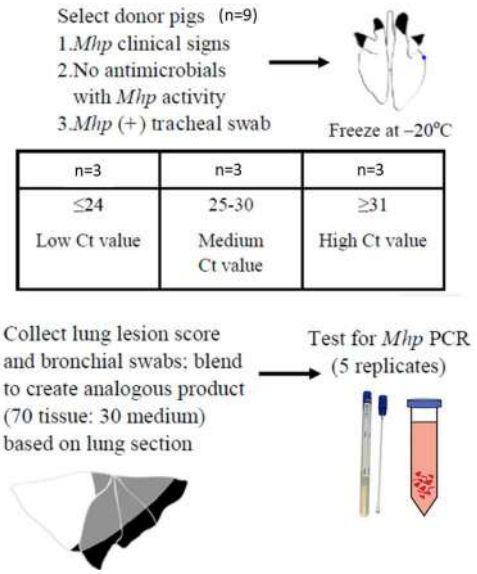

Figure 1. Experimental design of the study. Mhp: *Mycoplasma hyopneumoniae*.

Conclusions and Discussion

These results suggest the selection of donor pigs with low Ct values in DTC, as they correlated with greater and consistent bacterial load in the lung homogenate. In addition, our results indicate that all lung lobes could be employed for lung homogenate preparation, regardless of the presence/absence of lesions.

Acknowledgments

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A comprehensive investigation of pig lameness associated to *Mycoplasma hyosynoviae*

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Introduction

Since first recognized as a causative agent of arthritis in 1970 (1), *Mycoplasma hyosynoviae* (*M. hyosynoviae*) has become an increasing concern to swine producers by decreasing performance, affecting welfare and increasing the costs associated to the use of antimicrobials. *Mycoplasma hyosynoviae* has been also detected in joints with no apparent clinical lameness or macroscopic joint lesions (2, 3). Thus, it is still difficult to correlate diagnostic data to the actual disease prevalence in the field.

The aim of this work was to investigate the potential association between detection of *M. hyosynoviae* in oral fluid (OF) and synovial fluid (SF) samples and the clinical presentation of lameness in growing pigs.

Materials and Methods

Five wean-to-finish farms reporting recent history of lameness were enrolled in the study. Oral fluids were collected by pen (9 to 10 samples collected by site). Real-time PCR was performed on each OF to detect *M. hyosynoviae*. Lameness scores (0-4) were recorded in pigs from pens where OF were collected, as previously described (2). In addition, three finisher pigs, one healthy pig (score 0) and two lame pigs (score ≥3), were identified at each farm and euthanized to collect synovial membrane and SF samples from multiple joints, which were evaluated by histopathology and real-time PCR, respectively. The primers and probes were synthetized based on the Standard Operating Procedures (SOP) routinely followed at the University of Minnesota, Veterinary Diagnostic Laboratory for *M. hyosynoviae* detection (UMN-VDL SOP .0078). Samples were considered positive to *M. hyosynoviae* by real-time PCR when Ct ≤ 37. Real-time PCR results as well as histopathology variables were used for tests on differences between lame and healthy pigs by Fisher's exact test.

Results

Mycoplasma hyosynoviae was detected in all OF from the five enrolled farms. However, lameness (score ≥2) was observed in 4.42% of the assessed pigs across all five sites. Table 1 shows the results obtained by histopathological evaluation and *M. hyosynoviae* real-time PCR. Nine out of the 10 clinically lame pigs were positive to *M. hyosynoviae* in SF, at least in one of the sampled joints. From healthy pigs, three pigs tested positive to *M. hyosynoviae* in at least one of the joints sampled. Lameness scores were not correlated with detection of *M. hyosynoviae* in OF and/or SF. Detection of *M. hyosynoviae* in SF was also poorly correlated with histological alteration of the synovial membrane, as positive results were obtained in joints with no microscopic lesions and *vice versa*, both in lame and healthy pigs

Table 1. Contingency table displaying the number of individuals scored either lame or healthy (non lame) and the correspondance to *M. hyosynoviae* real-time PCR results and histopathological evaluation.

	Synovial fluid Real-time PCR	
	Positive	Negative
Lameness	9	1
No lamenss	3	3
Fisher value	0.1181 ($p < 0.05$)	

	Synovial membrane histopatology	
	Positve	Negative
Lameness	8	2
No lameness	5	1
Fisher value	1 ($p < 0.05$)	

Conclusions and Discussion

Despite the understanding of the clinical disease, the true prevalence, incidence, and dynamics of infection of *M. hyosynoiviae* remain unclear. Our results suggested that detection of *M. hyosynoviae* in SF as well as in OF samples was not necessarily associated with the presentation of clinical lameness, neither at the individual nor at the population levels.

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Characterization of *Streptococcus suis* isolates from various anatomic sites of diseased and healthy pen-matched control pigs using coagglutination and PCR-based serotyping methods

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Introduction

Streptococcus suis is a significant cause of mortality in the global swine industry. An important step in disease management is to identify the disease associated serotypes. Limited information is available concerning the serotypes associated with *S. suis* meningitis in the United States swine herd. The objective of this study is to characterize *S. suis* isolates from pigs with *S. suis* meningitis and healthy pen-matched controls using coagglutination (aggl) and PCR-based serotyping (mPCR)[1].

Materials and Methods

Twenty-four pigs between 3 to 4 weeks of age originating from seven different farms were included in the project. Twelve pigs had histological lesions compatible with *S. suis* meningitis and 12 pigs were healthy pen-matched controls. During necropsy, swabs of the nasal cavity, tonsil, brain, cerebral spinal fluid (CSF), joint fluid, bronchoalveolar lavage (BAL), ileum and apex of the spiral colon were aseptically collected for bacterial isolation. Alpha hemolytic colonies with different morphologies on blood agar were selected and confirmed to be *S. suis* using Matrix Assisted Laser Desorption/Ionization - Time of Flight Mass Spectrometry (MALDI-TOF MS). Nasal cavity and tonsil had isolation of up to four different isolates per animal. The isolates were serotyped by aggl test and mPCR. Reference strains (including 29 serotypes of *S. suis* as well as 6 serotypes of divergent *S. suis*) were used as positive controls[2, 3].

Results and Discussion

Numerous (232) *S. suis* colonies were isolated from the nasal cavity (38%), tonsil (38%), brain (6%), BAL (5%), joint (4%), ileum (3%), CSF (2%), and colon (2%) of both diseased and healthy pigs. The serotype causing meningitis commonly varied between farms, and on one occasion between animals from the same farm. Serotype 1 was the most common cause of meningitis (7); however, these pigs, while originating from different farms shared a similar gilt source. Serotype 10 was identified as the cause of meningitis in two pigs from the same farm. Serotypes 4, 5 and 11 also caused meningitis in individual animals from the same (4 and 11) and a different farm. A moderate to high pure growth was isolated from the brain and/or CSF in pigs with meningitis. The same serotype causing meningitis was also isolated from diseased pigs from varying sites including the joint (8), nasal cavity (3), tonsil (7), BAL (3) and colon (1). The pen-matched controls had isolation of the disease associated serotype from the nasal cavity (4), tonsil (4), brain (3), ileum (1) and colon (1). In most instances in which a meningitis causing serotype was isolated from an anatomic site, aggl and mPCR identified these isolates as the same serotype. A diverse set of serotypes were isolated across all farms and pigs from the nasal cavity (14 serotypes) and tonsil (21 serotypes). Individual pigs carried a less diverse set of serotypes in their nasal cavity and tonsil (1 to 3 serotypes) with the exception of one animal that had 4 different serotypes isolated from the tonsil. Regardless of anatomic site or pig status, 42% of isolates serotyped by aggl agreed with serotype by mPCR. Only 7% of isolates serotyped by aggl did not match the serotype by mPCR, many of these isolates originated from the nasal cavity and tonsil. Ninety isolates were untypeable (UNT) by aggl and 80 by mPCR. Together nasal and tonsil isolates represented the biggest part of UNT isolates for aggl (87.7%) and mPCR (77.5%). The UNT isolates were divided into three categories; UNT by both methods (22%), UNT by aggl but typed by mPCR (16%) and typed by aggl but UNT by mPCR (12%).

Conclusions

While this study included a conservatitive number of pigs from seven different farms, the data suggest that a potentially more diverse set of serotypes than previously thought cause meningitis in the United States swine herd. The same serotype caused meningitis on three distinct farms sharing a similar gilt source suggesting that sows play an important role in *S. suis* ecology. Two affected animals from the same farm had different serotypes (5 and 11) causing meningitis, indicating that farms undergoing *S. suis* neurologic disease can have different serotypes involved. Both mPCR and coagglutination readily identified the *S. suis* serotypes causing meningitis. Once the disease causing *S. suis* is identified through histopathology, bacterial culture, and serotyping, healthy cohorts could be screened for colonization by the virulent serotype using nasal and/or tonsil swabs.

Acknowledgments

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Natural transmission and detection of *M. hyopneumoniae* in a recently exposed naïve population

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Introduction

Mycoplasma hyopneumoniae (M. hyop) continues to be a prevalent and economically important, respiratory pathogen in the swine industry (1). Introduction of replacement gilts is commonly practiced for genetic advancement and productivity of sow farms (2). Nevertheless, replacement gilts are perceived as a risk for pathogen entry due to their frequent and consistent introduction year-round. Blood and oral fluid samples are commonly tested to clear incoming replacement gilts for several pathogens, including *M. hyop.* However, diagnostic limitations exist with these sample types, particularly due to low sensitivity during acute stages of *M. hyopneumoniae* infection. Therefore, the objective of this study was to evaluate the natural transmission and detection of *M. hyop* infection post-introduction of an infected gilt to a naïve population.

Materials and methods

A naïve gilt was placed in contact with two age-matched, *M. hyop* experimentally infected gilts. Laryngeal swabs and deep tracheal catheter (DTC) samples were collected from the naive gilt every 3 to 6 days to monitor for infection. Once the gilt was confirmed *M. hyop*positive in both sample types, the naturally infected gilt was placed in contact with other 29 age-matched, naïve gilts for 8 weeks (initial 3% prevalence). At 0, 1, 2, 4, 6, and 8 weeks post-contact (wpc), DTC, laryngeal swabs, and blood samples were obtained, along with the collection of an oral fluid sample from the pen level. At 8 wpc, gilts were humanely euthanized followed by the collection of bronchial swabs and the evaluation of lung lesion scores.

Laryngeal and bronchial swabs, DTC, and oral fluids were individually tested for *M. hyop* by real-time PCR. Samples were considered positive with a ≤39 Ct value. Sera obtained from blood samples were tested for the presence of *M. hyopneumoniae* antibodies using an indirect ELISA test (Idexx). Samples were considered positive with a ≥ 0.4 S/P ratio. The *M. hyop*natural transmission rate (β) per week was estimated using a Bayesian logistic regression model (assuming a SI dynamic model). Moreover, differences in time-to-detection based on sample type was estimated using a Cox Proportional Hazard Regression model and log-rank test. A p-value <0.05 was used for statistical significance.

Results and discussion

The naïve gilt became *M. hyop* positive at 23 days post-contact with the two experimentally infected gilts, thus confirming development of a naturally infected gilt, which was later placed in contact with naïve contact gilts.

At 6 wpc, 3% of contact gilts were *M. hyop* positive in laryngeal swabs and DTC. At 8 wpc, *M. hyop* was detected in 3%, 17%, and 27%, of contact gilts using laryngeal swabs, DTC, and bronchial swabs, respectively. Oral fluids were negative for *M. hyop* at all samplings in the study, regardless of the presence of infected gilts in the group contributing to the sample. Antibodies were detected in the naturally infected gilt and one contact gilt at 6 and 8 wpc, respectively. Differences in time-to-detection based on sample type were identified (p=0.02). Moreover, β was 0.36 (95% CI: 0.15-0.51).

Conclusions

In this study, secondary *M. hyop* infections from one infected pig were initially detected at 6 wpc. Moreover, the ability to detect *M. hyop* was influenced by sample type during the early stages of infection. These results suggest that the use of pathogen specific protocols is needed to achieve high detection sensitivity and to avoid the introduction of potentially infected gilts into naïve sow farms. Moreover, extension of isolation length can help contribute to higher likelihood of detecting a recent introduction while reducing sample size and potentially cost.

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Modulation of *Mycoplasma hyopneumoniae* infection using multiple vaccinations in gilts

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Introduction

Mycoplasma hyopneumoniae (*M. hyopneumoniae*) is the etiologic agent of enzootic pneumonia in swine (1,2). *Mycoplasma hyopneumoniae* infections result in significant respiratory challenges in the industry. Vaccination for *M. hyopneumoniae* is commonly utilized, as reduction in bacterial loads and clinical severity in vaccinated pigs have been shown (3,4). The effect of *M. hyopneumoniae* vaccine on the infectious pressure and transmission across different populations has been minimally investigated (5,6). Therefore, the aim of this pilot study was to evaluate the effect of multiple vaccinations on *M. hyopneumoniae* infection and transmission in seeder and naïve gilt populations.

Materials and methods

Naïve gilts (n=36) were allocated to four experimental groups: 1) NVI (Non-vaccinated, infected); 2) VI (Vaccinated, infected); 3) NVN (Non-vaccinated, naïve); 4) VN (Vaccinated, naïve). At 5, 7, and 9 weeks of age, VI and VN gilts were vaccinated with a commercial bacterin for a total of three doses. At 11 weeks of age, VI and NVI gilts were inoculated with *M. hyopneumoniae.* At 28 days post-infection (dpi), VI and NVI seeder gilts were relocated and housed with either a NVN or VN gilt (1:1 ratio) for 14 days.

Blood samples, deep tracheal catheters, bronchial swabs, and lung lesions were collected and evaluated for *M. hyopneumoniae* infection. Differences in bacterial load and lung lesions among groups were evaluated using a Mann-Whittney t-test. Moreover, the force of infection (λ) and incidence rate were estimated to determine differences in transmission among groups.

Results and discussion

At 28 dpi, 100% and 80% of NVI and VI seeder gilts were PCR positive and all vaccinated gilts were seropositive. Lower relative *M. hyopneumoniae* bacterial loads were identified in VI compared to NVI seeder gilts ($p<0.05$). Lung lesions were numerically lower in VI than in NVI seeder gilts ($p=0.78$). No transmission events were observed between VI and VN gilts, compared to other groups (i.e. 1-2 transmission events/group). Numerical differences in mean λ (0.00-0.05) and incidence rate (0.00-0.03) were observed among groups. Mean rate at which a naïve gilt became infected per week and incidence rate was lowest when both seeder and naïve gilts were vaccinated.

Conclusions

In this study, the implementation of multiple vaccinations against *M. hyopneumoniae* significantly reduced the relative bacterial load in vaccinated seeder gilts compared to their non-vaccinated counterparts. Moreover, numerical differences in *M. hyopneumoniae* transmission was observed across the four different seeder and naïve gilt populations. Results from this investigation provided insight in the potential impact of multiple vaccination on *M. hyopneumoniae* infection modulation. Further research encompassing larger populations is necessary to validate findings.

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Effect of tulathromycin treatment on *Mycoplasma hyopneumoniae* detection and infectious potential

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Introduction

Mycoplasma hyopneumoniae (M. hyopneumoniae), the etiologic agent of enzootic pneumonia (1,2), continues to cause significant economic losses in the industry worldwide (3). Control and elimination of *M. hyopneumoniae* with the use of management, vaccination, and antibiotics have been well-described (4). However, challenges in confirming *M. hyopneumoniae* elimination exist, due to detection and diagnostic limitations (5), as well as chronicity and persistence of infection. Due to limitations in *M. hyopneumoniae* bacterial culture, veterinarians have been challenged with the detection of *M. hyopneumoniae* by PCR long after antibiotic treatment, thus raising the question if the bacterium is still infectious. The study objective was to assess the effect of tulathromycin on *M. hyopneumoniae* detection and infectious potential during acute and chronic phases of infection.

Materials and methods

Transmission of *M. hyopneumoniae* was evaluated during the acute and chronic phase of infection. For each infection phase (acute and chronic), one age-matched naïve gilt was placed in contact with one *M. hyopneumoniae* seeder (1:1 ratio) that was either treated with tulathromycin, treated and *M. hyopneumoniae*-vaccinated, or non-treated for two weeks. Four replicates per treatment group were performed for each infection phase (n=24).

Seeders were intratracheally inoculated with *M. hyopneumoniae* strain 232 at a concentration of $1x10^5$CCU/mL. Gilts selected to be treated received two doses of tulathromycin (2.5mg/kg, IM) at either 8 and 18 days post-infection (dpi) or 64 and 74 dpi. Vaccinated gilts received 2mL of a commercial *M. hyopneumoniae* bacterin at 0 and 23 dpi.

Blood samples, deep tracheal catheters, bronchial swabs, and lung lesions were collected and evaluated for *M. hyopneumoniae* infection. Differences in transmission among groups were estimated by calculating force of infection (λ) and incidence rate.

Results and discussion

Acute phase of infection:

A numerical reduction in *M. hyopneumoniae* bacterial load was measured in treated seeders (0.5-3 Ct value difference) compared to those non-treated throughout experiment. Treated seeders had 16-18% less lung lesions compared to those non-treated. All naïve gilts remained *M. hyopneumoniae* negative when housed with treated seeders. For treated and vaccinated and non-treated groups, 1 and 2 transmission events occurred, respectively. Numerical differences in λ and incidence rate were observed between groups.

Chronic phase of infection:

At the end of the experiment, 50% and 33% of treated and treated and vaccinated seeders became *M. hyopneumoniae* negative. Moreover, a numerical reduction in *M. hyopneumoniae* bacterial load was measured in treated seeders (3 Ct value difference). On post-mortem, all non-treated seeders were *M. hyopneumoniae* positive via bronchial swabs compared to other groups. Differences in lung lesions were not observed between groups. All naïve gilts remained *M. hyopneumoniae* negative in all experimental groups.

Conclusions

Administration of tulathromycin modified *M. hyopneumoniae* infectious potential by reducing lung bacterial loads and the rate of new infections. Increased variability in *M. hyopneumoniae* detection was evident during chronic phases of infection. Further research focused on the characteristics of *M. hyopneumoniae* detection post-treatment is still necessary.

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Detection of antibiotics miss dosing and wrong use in commercial farms by using a real-time health-control plataform based on cell-phones

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Introduction

New technologies applied to animal health production facilitate the development of new applications for data collection and analysis (1). Moreover, livestock policies are focus on reducing the use of antibiotics (2). A novel control system has been developed to monitor health status of pigs, and in consequence, improving efficiency and reducing healthcare costs of pig farms.

Materials and Methods

The data entry is performed by using a web app (SaniTRAX, PigCHAMP Pro Europa, Segovia, Spain) which allows registering specific information of the health parameters from a specific batch of nursery or of fattening pigs using standard cell phones.

First, a database is set containing all the drugs used in the farm. In each batch of animals, the caregiver registers all health incidences observed. In particular clinical signs, medications and mortality, all of them by pathology, are daily recorded.

Parameters recorded about the use of a drug include the number of animals treated, doses per animal and target disease of the treatment. The system automatically calculates the animal daily doses (ADD; dose per animal and day calculated by means of recommended dosages and estimated live wights) and the used daily doses (UDD; dose per animal and day prescribed by the veterinarians). From this values, the UDD/ADD ratio is also calculated, which indicates if there is a gap between the recommended and the really applied doses. Values of UDD/ADD ratio above one means overdosing, while values below one means underdosing.

In the present work, preliminar outputs from the SaniTRAX system are presented, including data from 48 nursery batches (30,626 piglets in total) from 3 different commercial swine farms (Segovia, Spain).

Results

Global data from all 3 farms indicated the distribution of different pathologies affecting the nursery phase based on medications and on mortality. Based on the use of treatments, 40.0% of antibiotics were oriented to treat enterical pathologies, while 36.4% were used to treat respiratory diseases. Based on mortality, 44.3% of mortality was due to enterical diseases and 29.7% was "Others".

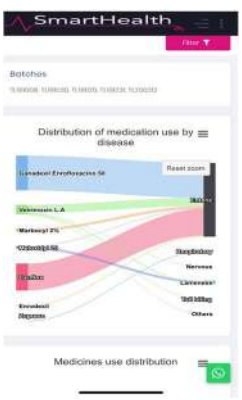
Meloxycam, dihydrostreptomycin, dexamethasone and benzylpenicillin showed the highest UDD/ADD ratio (≥1.5), indicating that these antibiotics are normally overdosed (Table 1). On the other hand, amoxicillin and enrofloxacin showed the lowest ratio (0.5 and 0.7, respectively), and then both are underdosed.

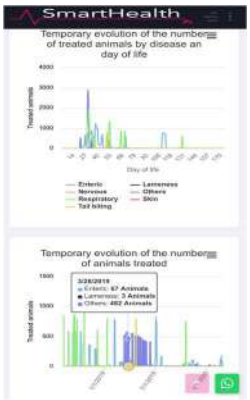
Table 1. Dosage ratio by medicine.

Active ingredient	Ratio (UDD/ADD)	Situation
Benzylpenicillin	1.7	Overdosed
Dexamethasone	1.7	Overdosed
Dihydrostreptomycin	1.7	Overdosed
Meloxicam	1.8	Overdosed

The higher use of antibiotics occurred during first two days after weaning when a high percentage of pigs showed digestive disorders. The mortality peaks occurred at 47, 50 and 55 days of life, and was also mainly associated to digestive pathology.

Besides, the app provides visual information of the success using different antibiotics for the treatment of the same pathology and clear information of the time evolution treatments performed, and treatments used for every disease (Figures 1 and 2).





Figures 1 and 2. Over time evolution of animals treated and dosing.

Discussion and conclusions

The use of a real-time plataform can solidly supports the decision-making process about health control and medicines use without physical presence on the farm.

The user can know the incidence and prevalence of the pathologies present in the farm and its distribution over time. Information about the convenience of using a specific medicine or active ingredient as well as withdrawal periods warning it is of great importance to ensure the efficiency and quality of the pigs produced.

Acknowledgements

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Increase of *Actinobacillus suis* detection in Brazil from pig farms

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Introduction

Actinobacillus suis (*A. suis*) is an important cause of respiratory diseases in swine with a higher prevalence in young pigs (1). The condition related to *A. suis*, in general, is observed in weaning piglets. Yaeger (2) listed three clinical forms caused by *A. suis*. The acute septicemic form that frequently leads to sudden death. The macroscopic lesions consist of petequial hemorrhage in multiple organs with fibrinous exudation at thoracic and abdominal cavities. In some cases, pleuritis, pericarditis and arthritis with multifocal abscesses in several organs can be observed. The histopathology lesions consist of neutrophilic inflammatory infiltrate with necrosis in multiple organs associated with intralesional bacteria. The second is the respiratory form showing cough and fever (2,3). The third form is the chronic septicemia condition. The animals may exhibit lethargy, anorexia, fever and red rhomboid skin lesions, similar to erysipelas. Abortions and sudden death can occur (2,3).

In the diagnostic routine, the conditions related to *A. suis* infection in older animals are sporadic, however, it is observed the emergence of *A. suis* in the diagnostic routine. The aim of this study was to confirm the perception about the A. *suis* emergence in growing and finishing pigs as well the swine age groups affected by *A. suis* from 2017 to 2019.

Materials and Methods

Two hundred and thirty-seven samples submitted from 2017 to 2019 at Microvet laboratory with diagnostic confirmation through isolation and bacterial identification, PCR and histopathology for *A. suis* deposited in the internal information system (Microsis) were evaluated. The results presented in the database were analyzed and filtered with keywords looking for results of interest.

Results and discussion

The found results are described below (Figures 1 and 2). In 2019, there was a significant increase of *A. suis* cases with 167 isolates, while in 2017 was just 44 isolates and 2018, only 26 isolates (Figure 1). It was observed increase of isolation in older animals (between 71 and 185 days) 9 times in 2019 than 2017 and 25 times compared to 2018 (Figure 2). In 2019, 60% of confirmed cases were in animals between 71 and 185 days (Figure 2). There are few publications about *A. suis* cases in growing and finishing pigs and it has been increased over the years. One explanation is about the immunological status from new herds without exposition and production of antibodies on able time with low title in growing and finishing pigs due to vaccination of sows, turning late the appearance of clinical signs.

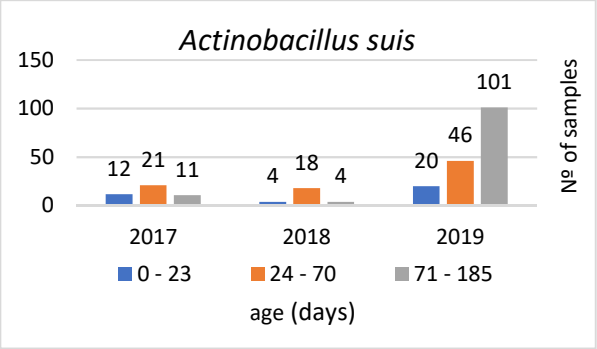

Figure 1. Number of positive results for *A. suis* beteween 2017 and 2019 stratified, in proportion, by age in days.

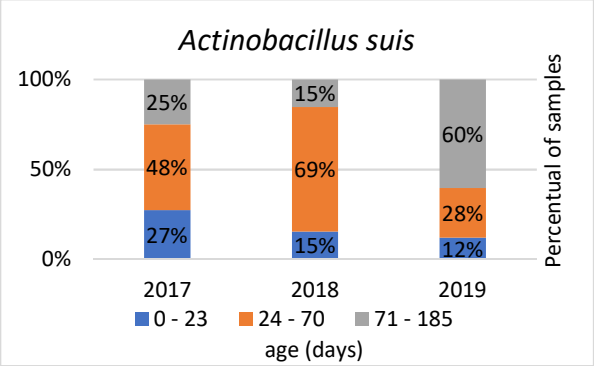

Figure 2. Percentual of positive results for *A. suis* beteween 2017 and 2019 by age in days.

Therefore, the perception of *A. suis* as a swine pathogen in growing and finishing pigs must be further studied. An alternative could be the vaccination of piglets post weaning as a vaccine booster to maintain the antibody title for *A. suis* in the final stages.

Conclusions

It was observed an increase in clinical cases related to *A. suis* in animals between 71 and 185 days of age, unrelated in previous years, bringing the importance of new methodologies for immunization to reduce the cases caused by this pathogen.

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Phenotypic and molecular characterization and antimicrobials susceptibility profile of *Streptococcus* spp. isolated from wild board in Rio Grande do Sul, Brazil

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Introduction

Streptococcus suis causes significant sanitary and economic losses in pig production worldwide, highlighting the meningitis cases in confined pigs. Nowadays, wild boars are also considered as reservoirs of potentially pathogenic *S. suis* for animals and humans (1,3). Therefore, the present work aimed the phenotypic characterization as well as the determination of the antimicrobial susceptibility profile (ASP) of *Streptococcus* spp. isolated from wild boars, focusing on *S. suis* molecular identification.

Materials and Methods

Twenty-six wild boars (10 females and 16 males) were slaughtered through official programs of wild boards population control, in the Rio Grande do Sul (RS) state, Brazil. Rectum, nasal and vaginal swab, fragments of kidney, liver, heart, spleen, lung, lymph nodes and tonsils were aseptically sampled and submitted to bacteriological culture on 5% blood agar in aerobic and microaerophilia at 37°C for 72 h. Next, 32 suggestive colonies of *Streptococcus* spp. were further analyzed at species level, using biochemical tests, hemolysis profile and CAMP test. The molecular characterization consisted of a PCR targeting *S. suis* and sorovar (SV) 1/2 as previously described (4). The disk diffusion method was used to evaluate the ASP of 32 isolates against 19 drugs according to the Clinical and Laboratory Standards Institute guidelines (CLSI).

Results

Around 80% of the *Streptococcus* spp. isolated were found in samples from the respiratory tract. Phenotypic tests allowed to identify 12.5% (4/32) *S. suis*, while PCR resulted in 34.4% (11/32), although none was positive for SV1/2. Thus, the remaining isolates (n=21) are possibly other species of *Streptococcus*. The ASP is described in Table 1.

Conclusions and Discussion

S. suis is an emerging zoonotic pathogen that should be considered as an occupational risk for people working in a wild boar environment (5). Reinforcing that, we demonstrated the presence of *S. suis* in 11 wild boars from 26 sampled. These animals are potential reservoirs of *S. suis* showing a high level of antimicrobial resistance profile. Increased resistance was observed against clindamycin, cephalexin, enrofloxacin, and tetracycline. Previous reports indicate ceftiofur, vancomycin, chloramphenicol and florfenicol as therapeutic options against infections caused by *S. suis* (2,6). Our results corroborate these findings since we observed more than 95% of sensitivity against these antimicrobials. On the other hand, 25% (8/32) of the analyzed isolates showed resistance to antimicrobials from seven classes, being classified as multidrug-resistant. These data are alarming since the microorganisms evaluated were isolated from free-living animals, never submitted to antimicrobial treatment. Finally, our findings reiterate the importance of monitoring wild boars since they are a potential source of zoonotic pathogens to humans.

Table 1. Antimicrobial susceptibility profile from *Streptococcus* spp. (n=32) isolated from free wild boars in RS, Brazil.

Antimicrobials	S (%)	I (%)	R (%)
Ampicillin	95,84	0	4,16
Penicillin	91,67	0	8,33
Cephalexin	66,67	0	**33,33**
Ceftiofur	95,84	0	4,16
Azithromycin	75	12,5	12,5
Erythromycin	70,84	16,66	12,5
Gentamicin	83,34	4,16	12,5
Enrofloxacin	50	25	**25**
Levofloxacin	87,5	12,5	0
Norfloxacin	87,51	4,16	8,33
Ciprofloxacin	100	0	0
Vancomycin	95,84	0	4,16
Trimetropim	91,67	0	8,33
Doxycycline	95,84	4,16	0
Tetracycline	83,34	0	**16,66**
Clindamycin	58,34	4,16	**37,5**
Sulfazotrim	91,67	0	8,33
Chloramphenicol	95,84	0	4,16
Florfenicol	100%	0	0

S: susceptible, I: intermediate and R: resistant.

Acknowledgments

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Effect of tulathromycin for controlling *Actinobacillus pleuropneumoniae* to reduce nursery mortality

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Introduction

Actinobacillus pleuropneumoniae (App) is the etiologic agent of pleuroneumonia in pigs and among the most important bacterial pulmonary pathogens in pigs and is found worldwide. App induces severe rapidly fatal fibrinohemorrhagic and necrotizing pleuroneumonia in naïve swine of all ages (1). The economic importance of App is principally due to the mortality, production loses and medication costs during acute outbreaks. The objective of this study was to evaluate the effect of nursery mortality after implementation of Draxxin® (tulathromycin) for controlling App.

Material and Methods

The study was conducted in a multi-site commercial farm with 12,000 productive sows, located in Easter Mexico. The farm has been positive to App since 2017 in the breeding herd, resulting in high mortality rates in nursery pigs due to a co-infection of App and PRRSV (Porcine Reproductive and Respiratory Syndrome Virus). In February 2018, the farm set up a metaphylactic medication strategy with Draxxin® (Tulathromycin) produced by Zoetis to control App in nursery pigs, implementing a medication routine program with 2.5 mg/kg at weaning (0.15 mL of Draxxin® per piglet) and 6 weeks of age (0.25 mL of Draxxin® per pig). Data from 2017, 2018 and 2019 were analyzed following the Statistical Process Control (SPC) Shewhart chart for nursery mortality at 10 weeks with a process change before (n=75) and after (n=58) the implementation of Draxxin strategy. In addition, a T-Test was performed to detect differences prior (PV) and after (AV) intervention (Draxxin® application). The SPC model and the graphs were realized through the Minitab® 18 software.

Results

Nursery mortality for PV was greater (P<0.001; 8.63%, UCL=17.63 and LCL= 0.37) when compared to AV (5.10%, UCL = 8.96 and LCL= 1.23) (Figure 1). This reduction in nursery mortality represents a decrease of 40.90% after the intervention (Draxxin® application).

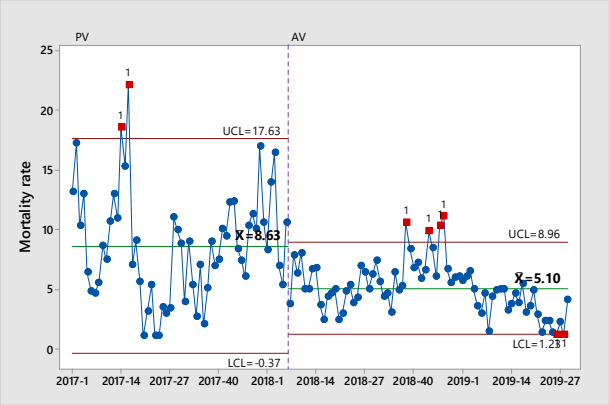

Figure 1. SPC chart of nursery mortality before and after administration of Draxxin.

Discussion and Conclusions

The Metaphylactic medication program applied in this farm was effective for decreasing the negative impact of high mortality rate in the nursery. Draxxin® metaphylactic intervention had an effect of mortality reduction, but further investigation is needed to evaluate the long-term effect in App infected pigs as part of elimination programs.

The common occurrence of PRRSV infection with bacterial infections, including *Actinobacillus pleuropneumoniae*, begs the question of the value of antibiotics for the treatment of the disease it causes. Tulathromycin is used for the treatment and prevention of respiratory disease in pigs, and it has been shown to exhibit immuno-modulating properties (2).

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The impact of *Haemophilus parasuis'* sow vaccination on growth performance and mortality level of nursery piglets under the pressure of very virulent PRRSV

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Introduction

Haemophilus parasuis (*H. parasuis*) or *Glaesserella parasuis* is the etiological agent of Glässer's disease, widely distributed all over the world as well as in Russian Federation (1). These bacteria colonize upper respiratory system of healthy piglets after birth and, and under certain conditions (for example, presence of PRRSV influence), invade the host and cause severe lesions (2,3). Systemic invasion is characterized by fibrinous polyserositis, polyarthritis and fibrinous meningitis that induce significant economic losses due to reduction in weight gain, necessity of wide antibiotic therapy and high mortality rate (4). Therefore, the aim of the study was to investigate the impact of sow vaccination against *H. parasuis* on growth and mortality level of nursery piglets under the pressure of very virulent PRRSV.

Materials and Methods

This research is a case report of one of the biggest swine producers in the Western part of Russian Federation (around 20 000 sows). It consists of 5 farms, infected by very virulent PRRSV (OB1_RU_2019_ORF5), which induced even sow deaths up to 15% and up to 40% mortality rate in piglets before 10w of age. The vaccination protocol against PRRSV based on mass vaccination with MLV vaccine (VP 046 BIS, Spain) 4 times per year. After three circles of mas vaccination the mortality rate among nursery piglets decreased to 12-15 %, but the pressure of the virus remains present, that confirmed by numerous ELISA and PCR tests. In that situation our main target was to decrease mortality level and increase ADG.

Due to this, autopsy of 196 died nursery piglets was made to identify the main cause of mortality. Histopathology based on standard fixation and staining protocol was also conducted for visualization of microscopic lesions.

Identification of *H. parasuis* was made by real time PCR from lung tissues through the determination of *infB* gene (QuantiTest PCRkit, QIAGEN). The presence of other frequent swine pathogens has been also detected by PCR.

The impact of *H. parasuis* was established by measurement of average daily gain and mortality rate before and after of saw's vaccination with Hiprasuis Glässer® (Spain).

Results

The mortality rate of piglets was rather high despite the implemented saws mas vaccination protocol against PRRSV and stable serology situation. That indicates the presence of another pathological factor as a main cause of mortality. During routine autopsy high level of polyserositis was established (table 1). Heart and lungs were the main affected organs.

Table 1. Frequency of pathological lesions.

Pathology	Lesions, %		
	+	++	+++
Polyserositis	10	40*	40*
Meningitis	0	5	0
Pneumonia	3	10	0
Polyarthritis	5	5	0

"+" – mild; "++"– moderate; "+++" – severe lesions (*p≤0.05)

The presence of these lesions was approved by histopathology, due to which many macrophages and neutrophils were present in pericardium, lungs and brain membrane, indicating acute form of bacterial infection. During PCR analysis *H. parasuis* DNA (Ct level = 21-27) was most often detected. That is why saw vaccination against *H. parasuis* based on standard protocol was provided. Despite the absence of changes in PRRSV serological status the mortality rate among pigs, that was born from vaccinated sows decreased more than twice (from 12-15% to 4.6-6.6 %) and feed daily gain raised from 280.1 to 454.7 g (up to 174.6 g per day) compare with piglets from unvaccinated saws.

Conclusions and Discussion

The improvement of productive parameters after vaccination of saws with Hiprasuis Glässer® indicates the high pressure of very virulent *H. parasuis* strain on the farm. The negative influence of pathogen was reduced by saws immunization and maternal antibodies action, which contribute the colonization of piglets' respiratory tract by commensal microbiota.

Therefore *H. parasuis* can make a great negative influence on growth and mortality level of nursery piglets masquerading behind the pressure of highly pathogenic PRRSV. Saws vaccination against the disease is an effective way to improve mentioned production parameters.

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Hipra Diagnose, Moscow, Russian Federation.

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Interaction between *Mycoplasma hyopneumoniae* and respiratory microbiome after natural infection of pigs at early stage

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Introduction

Mycoplasma hyopneumoniae (*M. hyopneumoniae*) is a swine respiratory bacterium causing significant problems in the swine industry [1]. *M. hyopneumoniae* has been shown to facilitate further infections with other respiratory pathogens [1]. Recent studies have explored the composition of sections of the swine respiratory microbiome [2-4]. However, a thorough evaluation of the respiratory microbiome during the early stages of *M. hyopneumoniae* infection has not been performed. Therefore, the objective of this study was to characterize the interactions between *M. hyopneumoniae* and the respiratory microbiome after natural infection of pigs at early stage.

Materials and methods

A natural *M. hyopneumoniae* transmission model was employed. Twenty-nine naive and one *M. hyopneumoniae* naturally infected gilt were allowed to commingle for 56 days and were humanely euthanized at the end of the study. Nasal, oropharyngeal, tonsillar, laryngeal, deep tracheal, and bronchial samples were collected from each gilt. DNA was extracted from the samples using PowerSoil Pro Kit (Qiagen). The detection of *M. hyopneumoniae* in the laryngeal, deep tracheal, and bronchial samples was evaluated through real time PCR. Microbiome composition was ascertained after sequencing of 16S rRNA V4 region. Reads were filtered and denoised using DADA2 pipeline in R 3.6.2 to obtain an amplicon sequence variants (ASVs) table. Inverse Simpson alpha diversity index was regressed on type of sample and *M. hyopneumoniae* status of the pig in a GEE model. Genus-level Aitchison beta diversity distance was regressed on type of sample and the interaction type of sample**M. hyopneumoniae* status of the pig in a PERMANOVA test stratified by pig. Finally, ASVs shown to be *M. hyopneumoniae* were regressed on the rest of the bacterial members of the respiratory microbiota in a LASSO model. The significance level was set *a priori* to 0.05.

Results and discussion

A total of 4,165 ASVs were detected in all samples. The five most abundant bacterial genera were *Streptococcus*, *Actinobacillus*, *Clostridium*, *Rothia*, and *Moraxella*, irrespective of *M. hyopneumoniae* status of the pig. These results agree with previous studies on healthy pigs [2, 4], but contrast with the previous findings in pigs with respiratory disease [2, 3] since the changes in the microbiome were not among the most abundant bacterial genera. The alpha diversity of the bronchial samples was significantly lower than that of the other sample types ($P<0.0001$) and was higher when the pigs were positive to *M. hyopneumoniae*, without reaching statistical significance ($P=0.0945$). Sample type explained 25.8% of the variation among the microbial composition of the samples (Fig. 1) and that effect was modified by *M. hyopneumoniae* status of the pig. This association between *M. hyopneumoniae* and respiratory microbiome was further interrogated at the ASV level. Bacteria from the genera *Bifidobacterium* and *Fretibacterium* were significantly negatively associated with *M. hyopneumoniae* after adjusting for type of sample ($P=0.013$ and $P=0.021$, respectively).

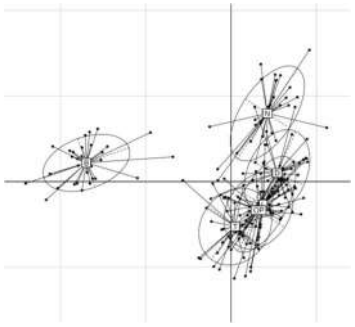

Figure 1. Plot of samples grouped by sample type after removing the pig effect. B: bronchial, N: nasal, D: deep tracheal, L: laryngeal, OP: oropharyngeal, T: tonsillar samples.

Conclusions

Results from this study showed the presence of interactions between *M. hyopneumoniae* and bacterial members of the respiratory microbiota during the early stages of infection. These interactions may have a role in deterring *M. hyopneumoniae* colonization and /or pathogenesis in pigs. Importantly, these interactions may change over time as *M. hyopneumoniae* can persist in infected pigs for up to 8 months, and this study was focused on early infection.

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Estimation of pool sensitivity for detection of *Mycoplasma hyopneumoniae* by PCR using deep tracheal catheter field samples

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Introduction

Mycoplasma hyopneumoniae (*M. hyopneumoniae*) is a cause of major economic losses to the swine industry, due to decreased growth, high feed conversion ratios, and high treatment costs in growing pigs[2]. Breeding herd eradication programs aim to eliminate exposure at the source, but diagnostic programs are needed to measure their success. Deep tracheal catheters have shown the highest reported sensitivity for detection of *M. hyopneumoniae* by PCR[8], but are an individual sample type. Oral fluids and processing fluids are common aggregate samples used in the swine industry[5,9]. However, oral fluid samples have shown low detection of *M. hyopneumoniae* by PCR[4], and more work is needed to validate processing fluids for detection of *M. hyopneumoniae*, as the source of the bacterium in the sample type is unknown and detection may be intermittent[7, 10]. Pooled-sample testing has been proposed as a more cost-efficient alternative than testing individuals when screening for low prevalence diseases[1]. However, because of the dilution effect, the sensitivity of the test when run on pooled samples is lower than its sensitivity when run on individual samples[3]. Therefore, the objective of this study was to determine the effect of pooling deep tracheal catheter samples on the sensitivity of real-time PCR for *M. hyopneumoniae* detection.

Materials and Methods

Deep tracheal catheter samples from a previous study[8], collected from pigs prior to inoculation and 113 days post-inoculation with *M. hyopneumoniae*, were used in the study. Samples collected from known negative pigs, prior to inoculation, were combined to form one homogenate negative sample. Individual expected positive samples (n=50) were pooled at ratios of one positive combined with two negatives and one positive combined with four negatives to form pools. Individual positive samples (n=50), pools of 1:3 (n=50) and 1:5 (n=50) were tested using DNA extraction (MagMAX™ Pathogen DNA/RNA Kit and KingFisher™ extraction robot, Life Technologies, Grand Island, NY, USA) a species-specific *M. hyopneumoniae* real-time PCR (VetMAXTM qPCR Master Mix and VetMAX™ *M. hyopneumoniae* Reagents Kit, Life Technologies, Grand Island, NY, USA). Samples with a Ct value <40 were considered positive for *M. hyopneumoniae*.

The sensitivities were calculated as the percentage of positive samples from the total samples using EpiTools along with the Clopper-Pearson (exact) confidence limits[6].

Results

DNA detection and pool sensitivity results are presented in Table 1.

Table 1. Diagnostic data and mean and 95% confidence interval of the individual and pool sensitivities for deep tracheal catheter samples at 113 days post-inoculation for *Mycoplasma hyopneumoniae* PCR.

Pool size	DNA detection[1]	
	# positive/# tested	Pool sensitivity (95% CI)
Individual	49/50	0.98 (0.89-0.9995)
3	42/50	0.84 (0.71-0.93)
5	41/50	0.82 (0.69-0.91)

[1]Real-time PCR. Samples with Ct values <40 were considered positive.

Conclusions and Discussion

Pool sensitivity decreased as pool size increased. There is a risk of not detecting one positive animal in a pool when the rest of the pool is negative. These samples represent the latest sensitivity reported after infection in the literature[8], and are the closest representation of pigs to sample at the end of an eradication program. The data have been applied to sample size calculations to detect *M. hyopneumoniae* in low prevalence scenarios, including the end of an eradication program.

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Leptospira spp. antibodies in wild boars (*Sus scrofa*) and hunters of Brazil

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Introduction

Leptospirosis is caused by *Leptospira* spp. and has been described as one of the most important zoonoses worldwide[1]. Although mostly reported in urban settings, rural areas also provide conditions for transmission and spreading, such as water accumulation, improper food storage, ineffective rodent control, presence of infected livestock and wildlife[2].

In Brazil, wild boars have been classified as exotic invasive species originated by Eurasian wild boars and hybrids, with nationwide hunting officially permitted by the Normative Instruction 03/2013[3]. Since previous studies have focused in *Leptospira* spp. antibodies of wild boars, no concomitant serosurvey has been reported to date in wild boars and hunters. Accordingly, the aim of the present study was to assess *Leptospira* spp. antibodies in wild boars and hunters of Brazil.

Materials and Methods

The study was conducted between October 2016 to May 2018 in rural areas in the Atlantic Forest of southern Brazil, including the unit conservation Vila Velha State Park, and in rural areas in the Cerrado biome of central-western Brazil, at the Aporé municipality. Free-range wild boars from degraded areas were sampled following slaughter by firearm, performed by legally registered hunters. Free-range wild boars from a natural area in the Vila Velha State Park were baited, photo-monitored, trapped and slaughtered by firearm (authorized by the Environmental Institute of Paraná- 30/17). Blood samples were obtained in wild boars by intracardiac puncture immediately after death, and in hunters by cephalic puncture (National Ethics Committee in Human Health-number 97639017.7.0000.0102). Serum samples from wild boars and hunters were screened for specific IgG *Leptospira* spp. antibodies by a microscopic agglutination test (MAT), adopting 100 as the cut-off titer.

Results

A total of 9/74 (12.2% - CI 95% 5.4 – 20.2%) wild boars, and none/49 (0.0%) hunter were seropositive for at least one serovar of *Leptospira* spp., with titers from 100 to 400.

Conclusions and Discussion

The seroprevalence of *Leptospira* spp. in wild boars herein was higher than in previous study with free-range wild boars in Brazil[4]. Anti-*Leptospira* antibodies were found in 8/19 (42.1%) wild boars of the state park. Wild boars may have been exposed to *Leptospira* spp. in overlapping environments with capybaras (*Hydrochaeris hydrochaeris*) and collared peccaries (*Pecari tajacu*), which may have a synergic impact on *Leptospira* spp. in-park occurrence. Despite no hunter has been seropositive to *Leptospira* spp. herein, hunters were the most exposed to zoonotic pathogens, including to *Leptospira* spp, when compared to other risk groups such as veterinarians, farmers and slaughterhouse workers[5]. Thus, the low hunter exposure herein may be associated to low environmental contamination in degraded areas. Thus, the findings herein suggest that hunters may be less exposed to *Leptospira* spp. infection than wild boars, particularly in natural areas.

Acknowledgments

Environmental Institute of Paraná and Coordination for the Improvement of Higher Education Personnel (CAPES).

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Development of an epidemiological surveillance platform for swine bacterial pathogens

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Introduction

Systemic swine bacterial agents, such as *Streptococcus suis,* remain a global challenge for production by reducing productivity, impacting welfare and promoting over-dependence on antimicrobials. The reduction in sequencing costs and improved efficiencies in bioinformatic pipelines have allowed whole genome sequencing (WGS) to be accessible in veterinary diagnostic laboratory settings, thus improving our knowledge on the molecular epidemiology of key pathogens.[1] *The objective of this initiative was to develop a surveillance platform for systemic endemic swine bacterial agents.*

Materials and Methods

Samples are submitted to the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL), and culture is attempted. For each isolate, date of isolation, state, flow, site, age, organ, clinical sign and clinical outcome is recorded. Genomic DNA is extracted using the ChargeSwitch gDNA mini bacteria kit. Multiplex whole genome libraries are prepared using the Nextera XT DNA kit with dual indexing. Genomes are sequenced using Illumina MiSeq platform. Pre-processed reads are assembled using SPAdes Genome Assembler. The following is extracted from each isolate: phylogeny, serotype, multi-locus sequence typing profile, putative virulence genes and antimicrobial resistance genes profiles. Phylogenetic trees based on the core genome are generated.

Results

The WGS database is comprised of roughly 800 contemporary clinical isolates (2016-2020) of *S. suis, G. parasuis*, *S. equisimilis*, *A. suis*, *M. hyorhinis* and *M. hyosynoviae*. Isolates originate from various tissue types and 45 swine systems (20 from the top 30 NA pork producers) (Figure 1). Three key pieces are integrated with this initiative; 1) the existent knowledge on the pathogens, 2) next generation sequencing technology and 3) effective collaboration with veterinarians who provide isolates and clinical information for each. Three main areas that can be refined using WGS pipelines; *pig flow management, disease prevention and surveillance*. The in-depth characterization of endemic agents from multiplier farms, has allowed veterinarians to determine the most appropriate source for their replacement gilts, and within a system improved multi-source commingling. Vaccine candidate selection for these agents has proven to be very inaccurate in the past. Using the expanded information afforded by WGS pipelines, the identification of optimal vaccine candidates can be vastly enhanced. It has also demonstrated how strains included in current autogenous vaccines from several systems did not align with isolates obtained from contemporary cases, evidencing that improvements in the vaccine selection process were warranted. Identification of AMR genes with corresponding clinical and susceptibility data can help identify suitable antimicrobials for use on farms and allows for monitoring of resistance genes. The distribution of certain AMR genes has helped to identify relevant strains. For surveillance, routine use of WGS data can help identify the emergence of new pathotypes or changes in prevalence within a flow. This data can be used in investigations to identify sources of introduction, risk factors and transmission routes, as well as support the elimination of disease -associated strains from pig populations.

Conclusions and Discussion

WGS for systemic bacterial pathogens is now offered as a routine service at the ISU VDL. The databases are maintained with routine submission of cases, and the most relevant pathotypes are included with each dendrogram to support epidemiological analysis. Current efforts are targeted towards the development of an online swine bacterial visualization, analysis and sharing platform that can be utilized by swine practitioners and diagnostic laboratories. Furthermore, continued inclusion of diverse strains from clinical and non-clinical cases will aid in the identification of global genetic markers, thus further improving rapid and accurate diagnosis for the disease-associated endemic bacterial strains in the field.

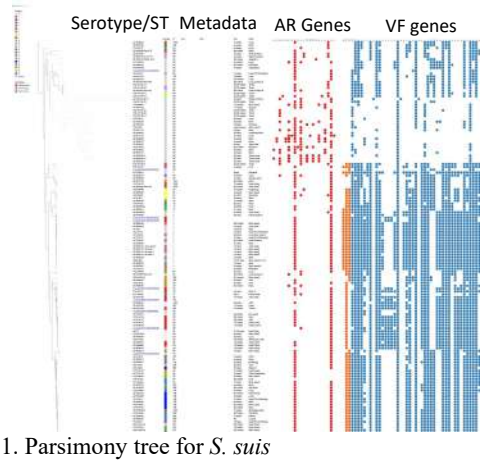

Figure 1. Parsimony tree for *S. suis*

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Serotypes and pathotypes of *Streptococcus suis* isolates from 2018 and 2019 in Brazil

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Introduction

Stretococcus suis is an important pathogen in swine production and is responsible for diseases in pigs from suckling to finishing stages (1). The objective of this study was to compile the information regarding *S. suis* isolates obtained from samples submitted to the Instituto de Pesquisas Veterinarias Especializadas (IPEVE) between 2018 and 2019.

Materials and Methods

IPEVE internal dataset was used to select cases that contained both *S. suis* isolation and serotyping from 2018 and 2019. Each case was classified according to the production phase of the sampled pig (suckling piglets: 0 to 28 days; nursery pigs: 29 to 70 days; growing pigs: 71 to 100 days, and finishing pigs: from 100 to 180 days), the pathogenicity of the isolate (2), and the serotyping results. The serotyping assay utilized was able to recognize serotypes from 1 to 14.

Results

One-hundred and sixty-nine cases fulfilled the requirements to be included in the data compilation. From those, 75 were obtained from 2018 and 95 were obtained from 2019. From the 169 *S. suis* isolates, 14.79% (25/169) were obtained from suckling pigs, 63.91% (108/169) from nursery pigs, 17.16% (29/169) from growing pigs, and 4.14% (7/169) from finishing pigs. Serotypes 1, 2 and 9 were the most frequent serotypes identified in both years (Fig. 1). Cross-reaction was identified in seven isolates (grouped as "others") and 46 isolates were nontypeable (Fig. 1). In regards of the pathogenicity of the isolates, 95.26% (161/169) were classified as pathogenic, while 4.74% were considered possibly opportunistic (8/169) (1). When classifying the isolates according to serotypes and pathotypes, serotype 2 and 4 were apparently more prone to include both pathogenic and possibly opportunistic isolates, while serotype 1, 3, 7, 9 and 14 were classified exclusively as pathogenic (Fig. 2).

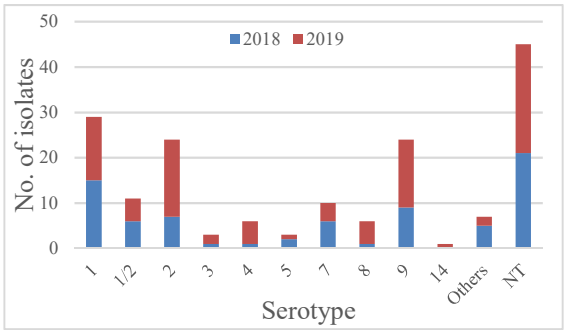

Figure 1. Distribution of *S. suis* serotypes isolates obtained from samples of sick pigs by year. Others and NT (nontypeable) represents isolates with serotypes that could not be differentiated by coagglutination.

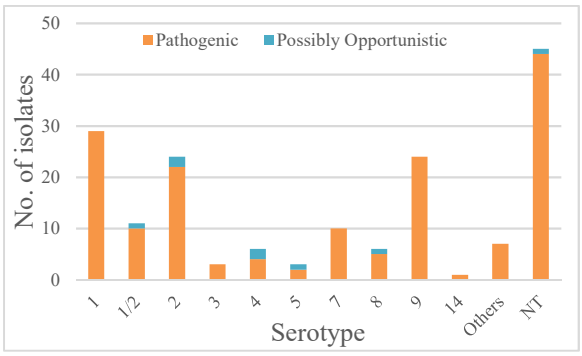

Figure 2. Distribution of *S. suis* pathotypes by serotype. The category Others and NT (nontypeable) represents isolates with serotypes that could not be differentiated by coagglutination.

Conclusions and Discussion

The most frequent *S. suis* serotypes in Brazilian herds in the past two years are serotypes 1, 2 and 9, while serotypes 2, 14 and 9 were the most frequent some years ago (3). Our results also point out that the predominant serotypes currently circulating in Brazil are different from the ones predominant in North America (serotypes ½ and 7) (2). The proportion of nontypeable isolates was relatively high (26.62% of the isolates) when compared to other suveys (1, 2, 3). This could be due to the fact that our serotyping method includes only serotypes 1 to 14, while there are up to 35 serotypes of *S. suis* described (4). However, it is still possible that some isolates were nontypeable due to the loss of capsular antigens.

These results justify additional research on the characterization of the *S. suis* strains circulating in Brazil to support better strategies for *S. suis* diagnosis and vaccine development to prevent *S. suis* impact in Brazilian pig herds.

Acknowledgments

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Genomic characterization and comparative analysis of *Lawsonia intracellularis* isolates

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Introduction

Lawsonia intracellularis is the causative agent of proliferative enteropathy (PE), one of the most common enteric infections in grow-finish pigs. Comparisons among isolates and characterization of potential bacterial subtypes using traditional typing methods have not been successfully applied due to the fastidious properties of this bacterium. These challenges have significantly limited advances on understanding the epidemiology of the PE. There are only approximately 20 isolates worldwide and the PE lab at the UMN has the most comprehensive collection of *L. intracellularis* isolates. The overall objective of this study is to characterize the genomic diversity among *L. intracellularis* isolates for future application in epidemiological studies and development of diagnostic tests.

Materials and Methods

L. intracellularis isolates were retrieved and co-cultured with murine fibroblast-like McCoy cells (1). Bacterial cultures were prepared for extraction and sequencing using DNA extraction using DNeasy Blood & Tissue Kit® (Qiagen). Extracted DNA was submitted to the University of Minnesota Genomics Center for DNA quantification and quality, Illumina library preparation with Nextera XT, and short-read sequencing on an Illumina MiSeq sequencer. The MiSeq generates 16 million reads of data per run where all bacterial isolates were multiplexed onto a single HiSeq lane, achieving approximately 400X coverage for each bacterial genome. Paired end read
technology, with a length of 300 base pairs, was utilized to facilitate genome assembly and single nucleotide polymorphism (SNP) detection. After removal of eukaryotic host sequences the remaining reads were assembled into contigs and scaffolds using MEGAHIT de novo assembler.

Results and discussion

Fourteen isolates, isolated between 1983 and 2016 from USA, Brazil and Denmark, obtained from pig, horse and hamster were used. Eleven isolates had at more than 80% of similarity when compared to the reference strain deposited in GenBank at a whole genome level, including the isolates obtained from the three different hosts. However, when considering the core genome, the number of SNPs was below 50 for all isolates, except from a horse, which had 3735 SNPs, when compared to the reference porcine strain (PHE/MN1-00). These significant genomic variances from the horse isolate were consistent in relation to allother 13 isolates, while the number of SNPs among all other isolates did not reach 40. These findings suggest that there are remarkable host specific genotypic traits in the horse isolate, as previously hypothesized (2). The lack of outstanding differences between the same isolates sequenced at low (18) and high (78) passages, which goes against to what is believed to occur to other bacterial species (3). We hence hypothesize that the change in pathogenicity between *L. intracellularis* isolates at low and high passages is potentially at gene expression level rather than mutation or loss of genes at DNA level, as it has been proposed to some bacterial species (3). The presence of a prophage in North-American isolates and in the horse isolate can also offer some explanation for the evolution and adaptation to the intracellular life-style of *L. intracellularis*. These hypotheses arecurrently being investigated and expected to be completed in the next few months.

Table 1. Number of SPNs among *L. intracellularis* isolates.

Isolates	Horse (US)	Pig Path (US)	Pig Non-path (US)	Pig (BRA)	Pig (DEN)
Horse (US)	-	3221	3221	3221	3232
Pig Path (US)	-	-	0	0	29
Pig Non-path (US)	-	-	-	0	29
Pig (BRA)	-	-	-	-	29
Pig (DEN)	-	-	-	-	-

US: United States, BRA: Brazil, DEN: Denmark. Path: pathogenic, Non-path: non-pathogenic

Conclusion

These preliminary data has shown that genomic difference among porcine isolates might not be substantial, even between a pathogenic (low passage) and a non-pathogenic (high passage) variant. Nevertheless, these results are critical for identifying genetic variation in order to differentiate field strains with focus on potential genotyping for diagnostic purposes.

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Evaluation of production performance from a 3000 sow-farm downstream growing pigs receiving a second dose of *Mycoplasma hyopneumoniae* vaccine at 10 weeks of age

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Introduction

Mycoplasma hyopneumoniae is the primary pathogen of enzootic pneumonia, a chronic respiratory disease in pigs, by destruction of the mucociliary apparatus, together with modulating the immune response, enhances the susceptibility of infected pigs to secondary pathogens[1,2]. Infections occur worldwide and cause major economic losses to the pig industry. The objective of this study was to evaluate production outcomes after implementing a second dose of Respisure-One® at 10 weeks of age compared to just one dose at 3 weeks of age.

Material and Methods

The present study was carried out in sites 2-3 (growing pigs facilities), of a multi-site farm located in Mexico, in these farms, at the end of 2016 and during 2017 signs and lesions characteristic of *M. hyopneumoniae* were observed confirming its presence by serology and PCR in clinical cases. In January 2018, the vaccination program with Respisure One begins at 10 weeks of age in pigs that were previously vaccinated at 3 weeks of age, therefore production outcomes defined as mortality, feed conversion, average daily gain and proportion of prime slaughter-weighted pigs were evaluated compared with previous groups. The evaluation was in a downstream pig flow of breed-to-wean 3000 sow-farm parity 1 (P1), which is part of a company of a 12,000-sow production system, this sow farm provides weaned sows to the rest of the farms in the production system, located in Western Mexico. Due to clinical signs and confirmatory diagnosis of M. hyopneumoniae between 12 and 20 weeks of age, the staff decided to reinforce *M. hyopneumoniae* immunity in the downstream pig flow from this P1-farm in 2018. These pigs were vaccinated at 3 weeks of age with a single dose vaccine combined with PCV2, the new intervention started with the first pig-batch of 2018, implementing full dose (2 ml) of Respisure-One at 10 weeks of age. Growing pigs closeouts (weaned to slaughter time) were captured for the evaluation. Fifty-two production closeouts were included in the analysis for 2017 and Fifty-one for 2018, representing before and after implementation of Respisure-One at 10 weeks of age.

Results

After the implementation of M.hyopneumoniae boosting strategy with Respisure-One , the farm's personnel observed fewer respiratory signs and lung lesions suggestive of *M. hyopneumoniae.*

These observations of respiratory improvement were reflected in a decrease of mortality and improvement of feed conversion and ADG as well the percentage of pigs that were sold with full value (prime slaughter weighted pigs). table 2.

Table 1. Closeouts information.

Year	Closeouts#	Pigs/Closeout (avg)
2017	52	1,216
2018	51	1,094

Table 2. Production outcomes from different Mycoplasma vaccination protocols. *(Variables that do not share a letter are significantly different. Fisher's exact test and two-sample T-test).*

Year	Mortality	F.C	ADG	PrimeWeightPigs
2017	16.5%[a]	2.5[a]	0.607[a]	86%[a]
2018	12.7%[b]	2.4[a]	0.646[b]	95%[b]

As additional observations, the farm is positive to PRRS virus and piglets are vaccinated at 10 days of age with PRRS MLV. During 2017 the main diagnosis was M. hyopneumoniae., during 2018, in addition to Mhyo, this site faced disease outbreaks of *Actinobacillus pleuropneumoniaen* (APP) and Porcine Epidemic Diarrhea virus (PEDv).

Discussion and conclusions

The effective control of *M. hyopneumoniae* depends on providing an optimal environment, including air quality, ventilation, temperature and adequate density. The use of a strict all-in-all-out production scheme1, as well as the adaptation of pre-entry replacements gilts. The sow farm under study presents a high replacement annual rate (>180%), this situation compromized, the effective control of *M. hyopneumoniae*. a booster strategy with Respisure-One helped to reduce clinical signs and increased productivity in this production system showing a statistically significant improvement in mortality, ADG and pigs sold with full value compared to previous closeouts. This intervention may be an option to improve the control of this disease where traditional vaccination programs for *M. hyopneumoniae* are being overcome due to high infection pressure.

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BIOSECURITY

Active regional surveillance for early detection of exotic/emerging pathogens of swine: A comparison of statistical approaches for selecting farms to be sampled

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Introduction

Farm selection plays a crucial role in livestock disease surveillance. In the past, farm selection was based on conditional random sampling, i.e., selecting farms using predefined criteria (stratified) or according to a random starting point with fixed intervals (systematic). However, the growing recognition of spatial dependence in disease spread (autocorrelation) brings the validity of these approaches into question. In this study, 5 statistically-based sampling methods were compared in terms of the probability of detection assuming 0.5% of farms were positive.

Materials and Methods

To conduct the analysis, the spatiotemporal spread of a contagious agent was modeled in a hypothetical region (195 × 300 kilometers in size) populated with 6,000 livestock farms (Figure 1). The disease spread model used in the study was derived from a model proposed by Ju et al. (2020):

$$\lambda_{f,t} = \lambda_{f-1,t} + \alpha \sum_{f' \in N_f} \exp(-d_{f,f'})\, p_{f',t-1} \quad (1)$$

where $p_{f,t}$ denoted the disease prevalence within farm f at time t where $f \in \{1, 2, \ldots, F\}$ *and* $t \in \{1, 2, \ldots, T\}$. N_f is the set of farms within 5 km radius of farm f. $\lambda_{f,t}$ indicated the odds of being infected $(0, \infty)$. α overall determined the degree of the spatial dependence from all other farms and $d_{f,f'}$ denoted the distance between farm f and f' $(f \neq f')$.

Using the model to simulate disease spread in the hypothetical region, farm selection based on simple random sampling (SRS) and 4 "spatially balanced" sampling methods were compared in terms of the probability of detection (prevalence = 0.5% of farms):

- simple random sampling (SRS)
- generalized random-tessellation stratified (GRTS)
- local pivotal method (LPM)
- spatially correlated Poisson sampling (SCPS)
- local cube method (LCUBE)

Notably, this study did not assess the efficiency of on-farm sampling and assumed that disease classification of farms selected for sampling was 100% accurate when on-farm prevalence was ≥ 5%.

Results

As shown in Figure 2, at a regional disease prevalence of 0.5%, SCPS and LCUBE had the highest probability of detection among six methods at specific sample size. For example, assuming 300 of 6,000 farms were sampled (5.0%), the probability of detecting ≥ 1 positive farm was > 90 % for SCPS and LCUBE, as compared to ~ 77% for SRS.

Conclusions and Discussion

This study showed that spatially balanced sampling methods may perform better than SRS, i.e., are more likely to select a positive farm. These results are consistent with Tobler's First Law of Geography: "Everything is related to everything else. But near things are more related than distant things." Thus, neighboring farms are more likely to have similar disease status than distant ones and the probability of infection and the distance to infected farms are inversely proportional. In contrast to SRS, the spatially balanced sampling methods used various approaches to adjust for spatial dependency. For that reason, the spatial balanced sampling methods required fewer samples than SRS to achieve the same probability of detection.

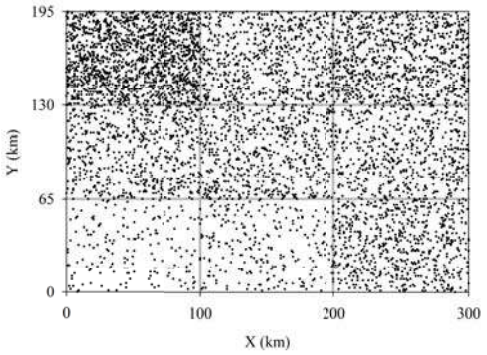

Figure 1. Distribution of 6,000 farms in a 195×300 km^2 hypothetical region. Note variable farm density, reflecting variation seen in production areas.

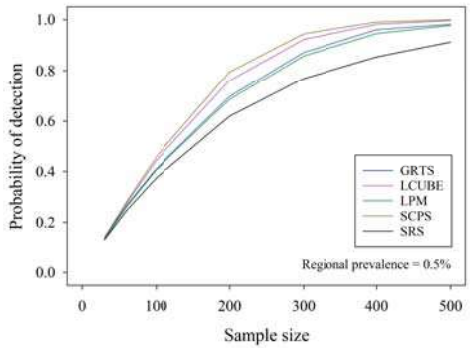

Figure 2. Probability of detection as a function of sample size and sampling method assuming 0.5% of farms were positive for the agent of interest.

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Validation of biofilm measurement indicators in water lines in pig farms and comparison of different pipe cleaning protocols

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Introduction

Regular maintenance of drinking water pipes is a key step to limit the development of biofilm, preserve the bacteriological quality of the water, the health of animals and the cleanliness of the equipment. Biofilm can act as a reservoir and can spread antibiotic resistance (1) and pig pathogens (2). This study aims to compare indicators that are easy to use in pig farming to determine the presence of biofilm and assess the effectiveness of different stripping protocols.

Materials and Methods

The trials were carried out on water circuits of post-weaning units of 30 French farms.
Several indicators were compared:

- Level of contamination by aerobic germs revivable at 22 °C (G22) and 37 °C (G37) in a water sample
- Measurement of Relative Light Units (LRU) by ATPmetry with swabbing of the internal surface of the pipeline (ATPsurf)
- Level of colouration of the water collected in a disposable boot cover (COUL): score 0 = clear water; 1 = coloured water
- Level of cleanliness inside the pipes as viewed by endoscopy (END).

Every biofilm stripping protocol was tested on 10 farms: FLUSHPIPE® (injection of water and air under pressure), HYDROCARE® (hydrogen peroxide stabilised by silver chelates) and an Alkaline (ALCANET®) - Acid (CID 2000®) protocol. The impact of all indicators was measured on samples taken at the end of the line befor e and after the cleaning protocol.

Results

Prior to the protocol, G22 and G37 significantly increased between the start and the end of the line, indicating an increase in the biofilm along the pipeline (Table 1).
All the indicators showed a significant reduction of biofilm after a stripping protocol (Table 1).
They allow to demonstrate the presence of biofilm and the effectiveness of a stripping protocol. None of the tested protocols was found to be statistically more effective than any of the others (Table 1).

Conclusions and Discussion

This study demonstrates the interest of several indicators to make farmers aware of the presence of biofilm and of the benefit of a stripping protocol.

Funding

This study was funded by the Ecoantibio Plan.

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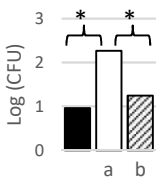
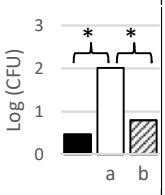
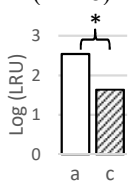
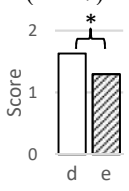
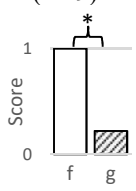
Table 1: Results according to the indicators studied and comparison of the effectiveness of the protocols

Results for the various indicators	**Legend** ■ Start of the line □ End of the line, before the protocols ▨ End of the line, after protocols	**G22** (N=26)	**G37** (N=24)	**ATPsurf** (N=28)	**END** (N=27)	**COUL** (N=9)
						
Success rate	FLUSHPIPE®	50% (n = 10)	40% (n = 10)	11% (n = 9)	22% (n = 9)	33% (n = 3)
	HYDROCARE®	30% (n = 10)	71% (n = 7)	40% (n = 10)	20% (n = 10)	100% (n = 4)
	ALCANET®- CID2000®	67% (n = 6)	71% (n = 7)	55% (n = 9)	62% (n = 8)	100% (n = 2)

Attempts to reduce LA-MRSA in pigs and in farm environment

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Introduction

Livestock-associated methicillin resistant *Staphylococcus aureus* clonal complex 398 (LA-MRSA) is wide spread in pig herds in most European countries. Most people working in positive pig farms carry LA-MRSA in their noses. These farm workers may transmit this organism to other people outside the farm premises and thereby jeopardize human health, especially in healthcare settings. If the level of LA-MRSA exposure to farm workers could be reduced significantly, this might also reduce the human transmission to the society.

The objective of this trial was to test if new disinfection technologies used extensively in a pig farm could reduce the level of LA-MRSA on the pigs and in the farm environment.

Materials and Methods

Four different disinfection technologies (T) were tested in a commercial finishing farm with an AI-AO flow by room (1). The four technologies were: T1) Constant recirculation of the room air in a closed system where the air was exposed to ozon and UV-light, T2) Electrolyzed water spayed over the pigs twice a week and added to the drinking water, T3) Reduction of airborne dust by at dust-binding product that was sprayed from nozzles in the ceiling over the pigs 2-5 times every 24 hours and T4) A disinfectant used before the pigs entered the room and sprayed over the pigs twice a week. All technologies but T3 had shown good killing rates of MRSA under small scale use or laboratory conditions.

T1, T2 and T3 were tested twice, whereas T4 was only tested once. At each test round data were collected from a treatment room (where the technology was applied) and one control room. Both rooms were run at the same time and allocated pigs originating from the same weaning section. Each room (493 m^2/1380 m^3) housed 500 finishers (30-100 kg) in 28 pens.

The amount of LA-MRSA in the finishers and environment were tested three times in each room: at entrance of the pigs, 4 weeks and 8 weeks after. MRSA was cultured from nasal swabs from 28 pigs (one per pen), from sedimented dust (2 samples per room) and in air-samples (two samples from a Sartorius air-sampler, sampling time: five minutes). The productivity of the finishers was registered (weight gain & mortality).

Standard laboratory methods were used for culturing and results were given as CFU per nasal swab, per g of dust, and per m^3 of air (1). MRSA-suspect colonies were verified as *Staph. aureus* by MALDI-TOF and the mecA gene of MRSA was detected by PCR.

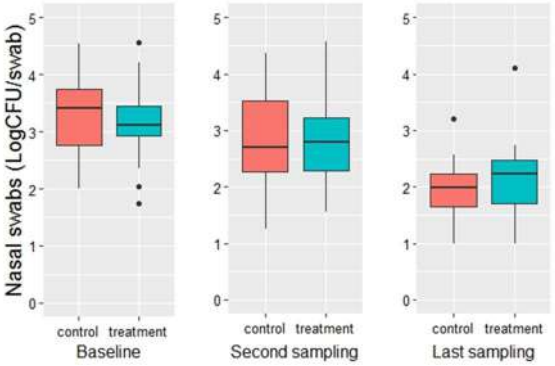
LA-MRSA loads and pig productivity were compared between test- and control-rooms for each technology.

Results

None of the tested technologies were able to reduce the level of MRSA in the pigs, in sedimented dust or in the air when compared to the controls, on all three sampling times.

There was a general trend for all technologies in all rooms (treatment & control) that the level of MRSA decreased through the 8 weeks sampling period (an example in Fig 1).

Fig 1. LA-MRSA-levels from 28 nasal swabs (LogCFU/swab) at the three samplings from the treatment and control room of T1.



As for the LA-MRSA levels, no differences were seen in productivity between finishers in the treatment rooms and the control rooms for any of the technologies.

Conclusions and Discussion

Even though pigs were exposed extensively to the different disinfection technologies, the level of MRSA in the pigs and in the environment, was not reduced. Once introduced, LA-MRSA seems difficult to eradicate from a pig farm.

Acknowledgments

The farmer and the companies supplying their technologies to the test are acknowledged.

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Investigating the association between on-farm worker movements and number of weaned pigs per sow

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Introduction

Even though it is recognized in the swine industry that fomites play an important role in the spread and maintenance of infectious diseases in swine herds[1], characterization and quantification of internal personnel movement patterns inside farms has never been previously explored. Such characterization could facilitate the identification of biosecurity breaches, focusing efforts to shorten disease duration, facilitate disease elimination, and improve disease prevention.

The goal of this study was to use an internal biosecurity system to investigate whether an increase in commonly regarded "risky" movements was associated with the number of piglets weaned per sow (PWS), one of the most important production parameter of interest in breeding farms.

Materials and Methods

Three commercial swine farms were enrolled in this project for a period of approximately one year (March 2018 to April 2019). Farm 1 was a 1,400 sow farm with a finishing facility, with no showers and no filter; located in Indiana. Farm 2 was a 4,000 shower-in farm also located in Indiana, with no filter and that had one nursery room. Lastly, farm 3 was a 4,500 shower-in filtered sow herd located in Iowa that had two nurseries on site. Wireless internet services were optimized throughout the farms and sensors were placed in different farm areas, including farrowing rooms, loading docks and nurseries/ finishers. These sensors were set up to detect Bluetooth-based beacon devices, which were individually distributed to farm employees. A movement was defined when an employee spent at least two minutes in one room from another room on the farm. Weekly movement and production data were gathered for analysis.

Statistical analysis was performed in STATA-IC 14. Linear regression models were created for each farm separately; followed by a linear mixed model with random effects for farms. For all models, the outcome investigated was the weekly number of piglets weaned per sow. Predictors of interest included three commonly considered "risky" movements, including movements from nurseries/ finishers to farrowing rooms, movements from shipping points (loading docks) to farrowing rooms, and movement between farrowing rooms. Confounding variables considered included season, number of piglets weaned per sow in the previous week and pre-weaning mortality. Statistical significance was declared at $P < 0.05$, and a tendency was declared at $0.05 \leq P < 0.10$.

Results and Discussion

A total of 56, 39 and 52 weeks of data was gathered for farms 1, 2 and 3, respectively. The mean (SD) number of piglets weaned per sow per week was 11.68 (0.52), 10.98 (0.48) and 12.39 (0.31) for farms 1, 2 and 3, respectively. Findings from statistical models revealed that, for farm 1, there was a decrease in approximately 1 piglet weaned for every 2 sows in the Winter compared to the Fall ($P = 0.008$). Furthermore, an increase in movements from the shipping point to the farrowing rooms decreased PWS by 1 pig for every 2-3 sows ($P = 0.02$). For farm 2, a 50-movement increase from the nursery room to the farrowing rooms decreased PWS by approximately 1 piglet per sow ($P = 0.03$). Farm 3 yielded similar findings, with a 100-movement increase from nurseries to farrowing rooms decreasing PWS by approximately 1 piglet per sow ($P = 0.04$). The final mixed model (combining all farms) showed that an increase in movements between farrowing rooms was associated with a decrease in approximately 1 piglet per 10 sows ($P = 0.03$), and that an increase in movements from grower pig rooms to farrowing rooms tended to decrease PWS by a similar amount ($P = 0.12$). All models accounted for production in the prior week, season, and pre-weaning mortality; a 'proxy' variable for treatment and potential disease challenges, which could be an important confounder. Our results suggested that excessive movement between farrowing rooms facilitates pathogen transmission; and confirmed that nurseries, finishers, and load out reas, commonly considered 'hotspots' for pathogens, can be particularly problematic[2].

Conclusion

This study showed that beacon-sensing technology can be implemented under commercial conditions to quantify and assess on-farm movements that may lead to biosecurity breaches, helping with disease control and elimination.

Acknowledgments

MSD Animal Health provided funding for this study, and PigCHAMP Pro Europa provided technology support for B-eSecure. The authors would like to acknowledge veterinarians, owners and staff.

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Swine transit analysis in main regions of Minas Gerais state, Brazil, 2014

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Introduction

Brazil stands out as the fourth largest world pig herd and pork exporter (1,2). The relevance of national pig farming in the economic and social scenario at global levels is noteworthy. The activity is characterized by concentrating its ventures in country main regions, especially South and Southeast, to reduce production costs and facilitate the supply chain logistics (1).

The animal`s movements are intense, and the transit dynamics can be a risk factor for diseases entrance and spreading (3,4).

Minas Gerais (MG) state is responsible for the largest number of pig slaughtering among southeast states (1,2). The study objective is to characterize and analyze the pig traffic in MG state, with emphasis on the animals movement among the state`s main regions, also identifying the traffic purposes: slaughter, fattening and reproduction.

Materials and Methods

Data collection was performed through the Animal Transit Guides (GTAs), a mandatory document that accompanies all loads of live animals, whose information includes the animals number and traffic purpose in 2014.

The GTAs were stored in state database, Instituto Mineiro de Agropecuária (IMA), the official organization responsible for the animal health and inspection (5). The software Pajek 1.24 was used for network design.

Results

A total of 84,595 GTAs were issued in MG in 2014, corresponding to 7,263,066 pigs on movement. Which 95,13%, 6,909,309 animals, were destined to state counties. The remaining had other states as destination. Animals for fattening from other main regions or states, with no inferior health status, were most representative category among all activities (43%), followed by the slaughtering destination category (41,2%). The animals fattening destination (45,2%) of great number of pigs was Triangulo and Alto Paranaíba (TAP), main state regions. Thus, those are the most vulnerable to pathogens introduction. In intense traffic area, as well in growing herds, disease extension and spreading may be faster.

High ingoing levels in termination units have higher risk of contagious diseases introduction.

Belo Horizonte area, main state city, received the largest pig volume, representing 31,2% of total animal transit. 54,8% of those had the slaughter as destination. Another main region, Zona da Mata, absorbed 26,1% of reproduction category destination.

Conclusions and Discussion

The study allowed better visualization and characterization of animals transit purpose within the state. It is concluded that the main transit entrance was for fattening to TAP region, characterizing the farms vulnerability for pathogens risk entrance.

Also was concluded that the tools used by the epidemiological surveillance system state body, can help in the health risks descriptions and allow the development of mitigation actions. In addition the methodology used can be expanded to other regions of the country.

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Inspection and epidemiological surveillance measures to prevent the introduction of African Swine Fever in Minas Gerais, Brazil, 2018

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Introduction

The African Swine Fever (ASF) virus can remain in contaminated pork, which can be in kitchen and food leftover (3,4,5). Since this information, the final garbage disposal establishments (GDE) such as landfills and garbage dump, become important risk factor for surrounding pig farms. The aim of the study is to georeference and geoprocess the GDEs and the surrounding pig farms in Minas Gerais State, Brazil. Also identify the GDEs risk of unauthorized pig presence linked to surrounding pig farms. Brazil stands out as the world's fourth largest pork producer and exporter. ASF is considered extinguished in Brazilian territory. Diet based on food leftover, without specific treatment, is a forbidden practice in Brazil, because of the diseases risk of reintroduction and spreading (1,2). Minas Gerais state is the largest pig producer in southeastern region, embracing many counties.

Materials and Methods

Data collection was carried out through a checklist application form fulfilled by the official animal health service inspectors during a preventive action named as "ASF-Landfill-Task Force 2018". An individual tablet was used to register, photograph and record the information, in the official database. Geoprocessing was performed by Terra View 4.2.1 software (INPE, 2012).

Results

A total of 690 landfills were inspected by the official veterinary service in 743 municipalities in 2018, during 15-day period of task force. There were 53 counties with shared landfills with another ones. During the inspection dogs and vultures presence were verified. The illegal pig presence in landfills was verified in four municipalities, a total of 34 animals (Figure 1). The pigs were immediately removed during inspection. Measures were taken to curb the presence of pigs in landfills. The surrounding pig farms in 10 kilometers radius, were geoprocessed for monitoring, a total of 61 properties with 8.033 animals.

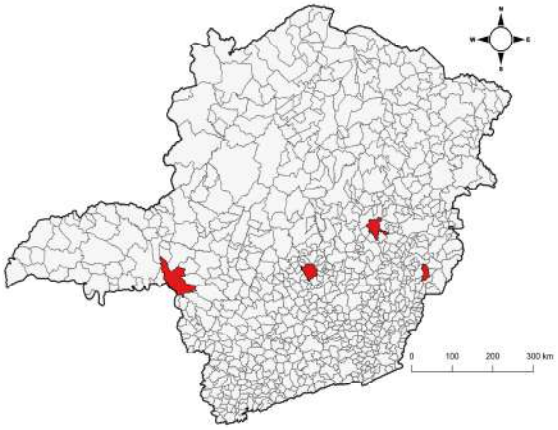

Figure 1. GDE in which illegal pig breeding was found. Municipalities with the illegal pig presence in landfills.

Conclusions and Discussion

All municipalities had the guidance of pig and other animal landfill entrance prohibition. Also was reinforced the illegality of feeding swine and other animals with food leftover.

The study allowed a better landfill characterization, including risky landfills and surrounding farms. The geoprocessing and inspection carried out by the official veterinary service from the epidemiological surveillance department, contributes with mitigation health risks actions for animal health and guarantee food safety.

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Efficacy of ALZOGUR® for treatment of pig slurry against *Brachyspira hyodysenteriae* in a simulated use test

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Introduction

The control of swine dysentery caused by *Brachyspira hyodysenteriae* is of vital importance for pig production due to substantial economic losses involved [1]. In this context, hygiene through proper disinfection is essential to avoid the spread of the disease between batches [2]. In addition, the selection of antibiotic resistant bacteria is not promoted by the use of disinfectants [3].

Present research aims to determine the efficacy of a commercial biocide, ALZOGUR® used in pig farms as disinfectant for inactivating *B. hyodysenteriae* in pig slurry via treatment of the residual liquid slurry, stored underneath the slatted floor, before new piglets are housed in.

Materials and Methods

Fresh pig slurry was collected from a commercial pig fattening farm that neither had been treated with antibiotics nor with long lasting bactericides during the fattening cycle preceding the sampling time. Slurry was contaminated using an inoculum of strain B204 (ATCC 31212) of *B. hyodysenteriae* at a final concentration of 10^7 bacteria/ml. In order to simulate the use for disinfection of liquid slurry in pig farms, contaminated slurry was distributed in vessels ensuring a height of 10 cm and test product was sprinkled to obtain a final concentration of 0.3% and a rate of 1 l/m^2 onto the slurry filled test vessels. After 1 h, additional water was sprinkled onto slurry filled test vessels, simulating rinsing, with 2 l/m^2. For negative controls, the same procedure was carried out using distilled water instead of product solution. Vessels were incubated during the procedure at 20 °C and under anaerobic conditions.

Determination of number of viable *B. hyodysenteriae* in test vessels at different times was estimated by counting the number of colony forming units per ml (cfu/ml). For each vessel, two subsamples were collected, from nearly the top and the bottom of test vessel. Both samples were mixed thoroughly before being plated on CVS agar plates (tryptic soy agar supplemented with 5% sheep blood and antibiotics). The plates were cultivated under anaerobic conditions at 38.5 °C for 72 hours. Duplicate samples were used for both treated and control vessels and two replicates were carried out per sample obtained.

Additionally, pH value was also determined in each slurry sample at the different times after the centrifugation of vessel for 10 minutes at 2000 g.

Results

The number of viable colonies of *B. hyodysenteriae* obtained from treated samples as well as from untreated controls at different times (0, 4, 8, 24, 48, 72, 96 and 120 hours) is shown in Table 1.

Table 1. Mean number of viable colonies (log cfu/ml) ± standard deviation obtained at different times for control and treated vessels

Exposure time [h]	ALZOGUR®	CONTROL
0	7.56 ± 0.060	7.48 ± 0.007
4	7.37 ± 0.157	7.12 ± 0.009
8	7.18 ± 0.063	7.11 ± 0.210
24	6.71 ± 0.189	6.90 ± 0.022
48	6.47 ± 0.096	6.43 ± 0.180
72	4.95 ± 0.450	6.50 ± 0.126
96	0	6.26 ± 0.023
120	0	4.57 ± 0.266

The pH of the slurry was in the slightly alkaline range and remained unchanged over the entire analysis period regardless of the treatment.

Discussion and Conclusions

According to the results of this experiment, ALZOGUR® has a bactericidal effect against *B. hyodysenteriae* in liquid pig manure after 96 hours of exposure. Hence, the data support the use of this product for disinfecting liquid slurry in pig farms, before new piglets are housed in, as it eliminates the risk of re-infection by *B. hyodysenteriae*. The use of effective disinfectants together with all-in/all-out management and strict biosecurity are relevant factors in the prevention and control of swine dysentery. However, further studies are needed to better understand the mode of action of this product.

Acknowledgments

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Forty-Three Critical Sampling Points for detecting ASFV in the environment during repopulation

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Introduction

African swine virus (ASFV) is a highly resistant viraemic virus. After ASF outbreak in the farm and several rounds of cleaning and disinfection, no detectable virus genome tested by Quantitative PCR (qPCR) in the environment is the precondition for repopulation in a swine farm. Hence, sampling from the right place is critical. For this purpose, a design methodology, called forty-three critical sampling points (43CSP), has been developed for the determination of critical sampling locations in repopulation farms.

Materials and Methods

The 43CSP considers disease outbreak place, humidity, sunlight, place hard for disinfection and easily forgotten factors. In total of 43 CSP were designed including pig barns, outside of barn, loadout chute, supply room, feed transportation center, living area, outside of the farm, etc. (Table 1). Six 43CSP case studies in different province of China were examined and compared with random sampling method. The detected ASFV genome qPCR positive ratio was compared and the most frequency found positive points were summarized.

Results

The 43CSP shows superior detective efficiency (13%, 42%, 27%, 36%, 85%, and 32% respectively) comparing with random sampling method (0%, 23%, 0%, 4.5%, 33%, and 17% respectively) in 6 case studies (Table 2). The most often positive place is water nipple (6/6), electricity panel (5/6), feeder (5/6), loadout chute (4/6) and compose area (3/6). These positive sampling points share one common character is that not easy clean and disinfection, or easily forgotten.

Conclusions and Discussion

This study demonstrated that the 43CSP could be successfully applied in all six case studies. It also suggests the high potential contaminated area in the pig farm, which has potential in applications for all China pig farms.

Table 1. Environment Critical Sampling Point (CSP) in swine farm

Functional Area	CSP	Sampling content
Inside of a barn	1. Aisle	Entrance, sags and crests
	2. Pen/crate floor	Four corners and middle
	3. Pen/crate handrail	Bottom of handrail
	4. Feeder	Bottom of feeder
	5. Pit	Four corners and middle
	6. Water nipple	Bottom and inside of water nipple
	7. Fan	Fan blade
	8. Water drop	Close to pig
	9. Wall	Close to pig
	10. Tool	Inside of different tools
	11. Electric panel	Surface and handle of electric panel
Outside of a barn	12. Chute for pig	Dirty floor and wall
	13. Chute for people	Dirty floor and wall
	14. Road	Dust/dirty aggregate place
	15. Truck	9 Points: driver cab, pedal, tire, bottom of truck, upper layer left top corner, upper layer right bottom corner, underlayer right top corner, underlayer left bottom corner, backboard
	16. Compose area	Dirty floor and wall
	17. Bury pit	Floor
Loadout platform	18. Clean/dirty line	Floor
	19. Dirty area	Dirty floor and wall
	20. Clean area	Dirty floor and wall
	21. Tool	Shared tools
Supply room	22. Floor	Floor
	23. Shelf	Shelf
Feed bin	24. Feed drain	Feed drain
	25. Feed leakage	Feed
Water	26. Water source	Water
Shower	27. Dirty area	Floor, closet
	28. Clean area	Floor, closet
	29. Grey area	Floor
People	30. Hand/hair	Hand, hair
	31. Cloth	Cloth
	32. Boots	Bottom of boots
Office	33. Floor/desktop	Floor, desktop, keyboard
Parking	34. Parking floor	Floor

Continue...

Gate Keeper	35. Foot pad	Foot pad
	36. Shoe changing	Bench bottom
	37. Register place	Register book and desktop
	38. Disinfection room	Floor, shelf
Gate entrance	39. Road	Road where pig truck went through
Outside road	40. Road	Road where pig truck went through
Wash/disinfection center	41. Road	Road where pig truck went through
	42. Power wash employee	Hair, hand, cloth, boots
	43. Washing machine	Surface and inside

Table 2 ASFV genome positive ratio tested by qPCR in environment

Farm	43CSP	Random sampling
Heilongjiang A	13%	0%
Hebei B	42%	23%
Henan C	27%	0%
Jiangxi D	36%	4.5%
Hunan E	85%	33%
Guangdong F	32%	17%

Estimating Senecavirus A seroprevalence and farm-level risk factors in the United States

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Introduction

Senecavirus A (SVA) is a viral agent associated with vesicular disease and neonatal mortality in swine herds in different countries[1]. In the United States (US), SVA has been previously reported but currently there are no estimates of the seroprevalence of this virus. Therefore, the objectives of this study were to estimate the proportion of sow and growing pig farms with anti-SVA serum antibodies and determine risk factors for seropositivity.

Materials and Methods

A cross-sectional observational study was conducted at the national level in the US. Data on SVA farm prevalence is scarce; therefore, it was decided to conduct the study to be 95% confident to estimate a 50% farm prevalence with a 10% allowable error. Based on this, 95 sow and 95 growing pig herds were needed to be selected for testing. Thirty samples were collected per farm, which allows us to be 95% confident in detecting at least one positive sample when the within-herd prevalence is at least 10%. Samples collected were tested through an indirect immunofluorescence assay (IFA) for anti-SVA antibodies in the University of Minnesota's Veterinary Diagnostic Laboratory. Herd sensitivity (94%) and specificity (100%) were highest when the number of positive samples needed to declare a herd as positive was 1, therefore, that was the cutoff used to classify herds as positive. Participating farms were also asked to answer a farm characteristics survey to determine herd-level risk factors associated with the presence of anti-SVA antibodies. Variables were screened initially for association with the herd status through univariate logistic regression models, and variables with p-values lower than 0.20 entered a forward selection process for the building of a generalized mixed model.

Results

A total of eight production systems and eight veterinary clinics participated in the study, including 34 swine veterinarians that were responsible for sample collection and shipment. Overall proportion of positive samples among sow and growing pig sites was 268/5762 (4.7%). Mean number of positive samples and standard deviation was 11.2 and 10.1 respectively in positive farms. Overall proportion of seropositive farms was 12.5% (24/192). There were positive sow and growing pig farms in 6 and 5 states respectively, out of 16 tested states. Among sow farms, the proportion of positive herds was 17/97 (17.5%) and the proportion of seropositive growing pig herds was 7/95 (7.4%). A total of 150 surveys from 192 farms (78.1%) were captured for the risk assessment analysis. Results from the multivariable logistic model can be seen in table 1.

Conclusions and Discussion

Results show that SVA is present in US pig farms across the country, but in a lower proportion than expected. The relatively high number of positive samples per positive farm rule out potential false positive results, and the randomized manner to which sampling was conducted adds to the robustness of the results. Farms that practice carcass rendering had 6.08 higher odds of positivity, which is known to increase the likelihood of having other diseases as the rendering truck goes from farm to farm playing a potential dissemination role. Employees staying in the farm after loading pigs appeared to have a protective effect, however, there is no clear explanation for this finding. Sow farms had 20.16 times higher odds for positivity, most likely due to their continuous flow nature, and for the higher movement of people, animals and trucks for activities such as gilt replacement, weaning piglets and culling sows. The practice of fewer biosecurity measures also yielded higher estimated odds, which highlights the importance of adopting measures for risk mitigation.

Table 1. Multivariable logistic regression assessing farm level risk factors for farm seropositivity to Senecavirus A in both sow and finishing farms, with company as a random effect. **Rendering*: compared to composting carcasses on site, burying and incinerating. ***Biosecurity measures*: shower in/out, bench system, use of farm-specific boots, use of farm-specific clothing, and downtime.

Variable	OR	p-value	95%CI
Rendering*	6.08	<0.01	1.73, 21.42
Employees stay in the farm after loading the pigs	0.14	0.03	0.02, 0.80
Sow farm	20.16	<0.01	3.47, 116.98
Less than 4 biosecurity measures count**	6.72	<0.01	1.64, 27.51

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CLINICAL CASES

Detection of Lung Pathogens Slaughter by PCR and its Effect in Average Daily Gain

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Introduction

Porcine respiratory disease complex (PRDC) is a multifactorial disease that causes important losses to the swine industry (1). Most common agents associated are: *Mycoplasma hyopneumoniae* (Mh), *Pasteurella multocida* (Pm), *Actinobacillus pleuropneumoniae* (Ap), *Haemophilus parasuis* (Hp) influenza A virus (IAV), porcine circovirus type 2 (PCV2) and porcine respiratory and reproductive syndrome virus (PRRS). However, the etiology of PRDC varies among countries or farms (1, 2). Slaughter check is a useful tool to analyze which agent are presented in a farm and to evaluate the severity of lung lesion.

The objective of this study was to analyze the relationship between lung lesion at slaughter, average daily gain during the fattening period and PCR detection of lung pathogens.

Materials and Methods

A 2000 sows, farrow-to-finish, one site farm was selected for the study. The farm is positive to Mh, Pm, Ap serotype 8, Hp, IAV and PCV2. Vaccination protocol was Mh and PCV2 (combined vaccine at 21 days old) and subunit vaccine against Ap at 40 and 60 days old. In addition, prophylactic pulses medication with florfenicol (40 ppm) lincomycin (88 ppm) and tylmicosin (400 ppm) were applied.

A batch of 40 pigs (22 castrated males and 18 females) were randomly chosen at 130 days old, weighted and ear tagged. At 180 days of life pigs were slaughtered, individual weight were recorded and average daily gain (ADG) was estimated. The lungs of all pigs were examined according to Christensen (3) and sampled. PCR studies were carried out (2). Differences in ADG and severity of lesion (% affected lung area) were analyzed by ANOVA ($P<0.05$).

Results

Most of the lungs (90%) showed lesion of bronchopneumonia or pleuritis. Interestingly, no positive samples to Pm, Ap or Hp were observed. In table 1 are presented the results of the analysis.

Table 1. ADG, affected lung area and pleuritis in relation to PCR results.

PCR +	N	% Affected lung area	ADG	Pleuritis (%)
Mh	13	9.923	1.091	62.5
PCV2	8	6.125	1.010	60.0
Mh/PCV2	12	8.166	0.961	50.0
Negative	7	10.000	1.085	40.0
Total	40	8.655	1.053	66.6

There were not statistical differences among groups in ADG and severity of lung lesion.

Conclusions and Discussion

The high percentage of Mh positive lungs was similar to previous studies (2). On the other hand, the percentage of PCV2 positive lungs was unexpected. This higher rate of PCV2 positive lungs could suggest a failed in vaccine process. However, no positive or suspected PCV2-SD was observed during the study.

The negative PCR results to Pm, Hp and Pm could be associated to the pulse medication used in the farm or the chronic stage of pleuritis observed at slaughter.

There was not a statistical difference in ADG among groups, however, double positive cases (Mh/PCV2) showed a decrease compared to the Mh group of 131 g/day in ADG. Also, PCV2 group showed the second lowest ADG group (<81 g/day compared to Mh group), detrimental effect of PCV2 in ADG in absence of PCV-SD was previously reported (1).

The results of this study showed the variability of the productive effect of individual pathogens in a farm context and highlight the importance of perform routine etiological studies to understand the respiratory disease present in a farm.

Acknowledgments

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Relationship between average daily gain and lung lesion at slaughter

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Introduction

Porcine respiratory disease complex (PRDC) results from a combination of infectious agents and environmental stressors (1). One of the most commonly used tool to analyze the impact of PRDC is slaughter checks of lungs (2, 3). Several studies have been reported the relationship among severity of lung lesions and productive losses, however, most of these studies were published 20 or more years ago (3, 4).

The objective of this study was to analyze the relationship of lung lesion at slaughter and average daily gain during the fattening period.

Materials and Methods

A 2000 sows, farrow-to-finish, one site farm was selected for the study. The farm is positive to *Mycoplasma hyopneumoniae* (Mh), *Pasteurella multocida* (Pm), *Actinobacillus pleuropneumoniae (*Ap) serotype 8, influenza A virus (IAV) and porcine circovirus type 2 (PCV2). Vaccination protocol was Mh and PCV2 (combined vaccine at 21 days old) and subunit vaccine against Ap at 40 and 60 days old. In addition, prophylactic pulse medication with florfenicol (40 ppm) lincomycin (88 ppm) and tylmicosin (400 ppm) were applied.

A batch of 170 pigs (85 castrated males and 85 females) were randomly chosen at 130 days old, weighted and ear tagged.

At 180 days of life pigs were slaughtered, individual weight was recorded and the lungs of all pigs were examined according to Christensen (2).

A simple linear regression was used to analyze the relationship between ADG and score of lung lesion $P<0.05$ was considered significant.

Results

Average daily gain of the group was 1039 g/d. Descriptive statistics of ADG are presented in table 1

Table 1: descriptive statistics of initial weight, final weight and ADG in each sex.

Sex	Initial weight	Age	Final weight	Age	ADG
Male	87.53	130	130.38	180	1045
Female	87.63	130	129.90	180	1031
Total	87.579	130	130.041	180	1038

A high percentage of lungs were affected by bronchopneumonia lesions (acute and chronic lesions). However, the mean lung area affected was moderate. Chronic pleuritic was observed in a high percentage of lungs.

The results of the slaughter check are presented in Table 2.

Table 2. Results of slaughter check in 170 pigs.

Type of lesion	%
Catharral bronchopneumonia (A)	12.94
Mean lung area affected type A	409
Complicated bronchopneumonia (B)	34.71
Mean lung area affected type B	8.86
Fissures (Scars)	38.24
Prevalence of bronchopneumonia	85.88
Mean lung area affected (A + B)	8.22
Fibrinous bronchopneumonia	0.00
Chronic pleuritis	32.35
Embolic pneumonia	0.59
Bronchopneumonia > 10%	15.88
No lesion	32.94

There was not statistical relationship between ADG and lung lesion score but a trend was observed ($p = 0.10$).

Conclusions and Discussion

Previous studies showed a relationship between lung lesion at slaughter and ADG. However, in our study only a trend was observed, the lack of relationship could be associated to the relative low number of pigs evaluated or the moderate severity of lung lesion observed. It is important to mention, that previous to this study the farm suffered an outbreak of pleuropneumonia due to Ap. In response, tylmicosin pulse was applied and the antimicrobial effect of this antibiotic could be partially controlled respiratory pathogens and reduced the severity of lesions.

The results of this study show the importance of evaluate the effect of pathogens and disease in field trials in current production conditions in order to determine the productive losses associated to it.

Acknowledgments

This study was suported by Biofarma SA and PICT 2015-1232.

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Salmonella Heidelberg in nursery pigs with diarrhea: first case report in Brazil

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Introduction

Salmonellosis is an endemic disease in pig farms that causes economic losses (1, 2). The main serotypes that usually cause disease in those animals are *S*. Choleraesuis and *S.* Typhimurium (3). However, the presence of other serotypes was worldwide described in pigs, such as *S.* Derby, *S.* Infantis and *S.* Heidelberg (4, 5, 6). Regarding *S.* Heidelberg in Brazil, it was only described in slaughterhouses and in feces of non-symptomatic finishing pigs (7, 8, 9). Therefore, this is the first case report of *S.* Heidelberg causing diarrhea in nursery pigs.

Materials and Methods

Three pools of pig feces were analyzed at the Bacteriology Laboratory of Escola de Veterinária e Zootecnia of Universidade Federal de Goiás, Brazil. Samples were collected and sent by a veterinarian who was attending a swine herd that had a diarrhea outbreak in nursery pigs - aged range 30 to 45 days.

We processed the samples according to the laboratory protocol based on technical standards from Brazil's Ministry of Health, Fundação Oswaldo Cruz (FIOCRUZ, Brazil) and the Nacional Health Sanitary Surveillance Agency (ANVISA, Brazil) for isolation and identification of *Salmonella* sp. Thereafter, isolates were sent for serotyping to the Enterobacteria Laboratory of FIOCRUZ in Rio de Janeiro.

Results

We isolated *Salmonella* sp. colonies in two fecal samples. These were sent for serotyping and *Salmonella* Heidelberg was identified.

Conclusions and Discussion

Isolates of *S.* Heidelberg in pigs are uncommon. However, this serotype is often reported in chicken and poultry products – meat and eggs, since 1982 in Brazil (10).

The pig farm where this salmonellosis outbreak occurred is located in the middle west of Brazil, in a city in which the economy is based in agribusiness, especially in pig and poultry farming. The short distance between farms raising different species and poor biosecurity measures may explain the outbreak occurrence.

S. Heidelberg is not adapted to only one host; therefore, it is also potentially pathogenic for humans. Human salmonellosis can occur by consumption of contaminated food or by direct contact with animals. Although these serotypes with a broad host range cause mild or subclinical infection, it is still a concern in public health (1, 2).

Cases related to *Salmonella* sp. in pigs have increased considerably in Brazil. Investigating the involved serotype is important for a complete epidemiological investigation and to plan strategies to prevent and control the infection. In this outbreak, the veterinary recommended improving facilities hygiene, apply sanitary break and the herd was treated with ciprofloxacin in drinking water at a dose of 10mg/kg BW for five days.

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Summary of prevention and control of African swine fever in the Xiaoshan area

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Introduction

African swine fever is an acute and highly contagious infectious disease of pigs caused by ASFV. Pigs of all ages are susceptible. Clinical features: high fever appetite to eliminate skin and internal organs bleeding. Xiaoshan is one of the more intensive pig breeding areas in Zhejiang province. Within the area of 70 square kilometers, there are 23 large-scale pig farms, 50,000 basic sows, and 550,000 hogs.

Materials and Methods

Through the means of epidemiological investigation and laboratory tests, the number of African swine fever infections and incidence in the Xiaoshan area was determined. The prevention and control of African swine fever in the Xiaoshan area can be divided into three stages. The first phase of the nervous phase, from August 2018 to October 2018. At this stage, the whole country began to appear African swine fever disease gradually, most of the pig farm owners in the Xiaoshan area did not know what was African swine fever and how to solve the problem. Only to strengthen the biosafety measures, such as enhanced disinfection, limited access to personnel, field closure management. The second phase of coordinated inter-regional prevention and control is from October 2018 to July 2019. At this stage, as many Xiaoshan pig farm owners owning pig farms in Jiangsu, where was at its peak in September, had a direct or indirect experience of African swine fever. Coordinated inter-regional prevention and control were started to block the transmission routes. All vehicles entering Xiaoshan will be sterilized at designated places. Disinfection equipment will be installed at the gate of the pig farm. Personnel access management and daily disinfection management will be more strict. The third stage is gradually infected to the onset of all infections from August 2019 to February 2020. The arrival of the typhoon rainy season and the rise in pig prices, coordinated inter-regional prevention, and control began to loosen and the docking of designated slaughtering could not be done. With the serious epidemic situation in the whole country, many large group companies came to Xiaoshan to purchase sows and weaned piglets on a large scale, the biosafety gradually appeared loopholes. With the increase of vehicles, the cleaning and disinfection of fixed point washing are not complete. After the live pigs take away, they need to go to a fixed place to issue the quarantine certificate. This place has not been cleaned and disinfected uniformly, which has become another loophole for epidemic prevention in the Xiaoshan area. After the occurrence of African swine fever positive pig farms, the public harmless treatment center and harmless treatment vehicle in the Xiaoshan area have become a new spread of the virus vulnerability.

Results

In the first phase, 23 pig farms were stable and no African swine fever virus-positive farms were found.
In the second phase, 23 pig farms remained stable, with no African swine fever virus-positive farms.
In the third stage, 23 pig farms gradually began to show positive African swine fever virus farms until all farms were positive.

Conclusions and Discussion

Coordinated inter-regional prevention and control and strengthening biosafety are effective means to prevent the infection of African swine fever. In the cleaning and disinfection of fixed point, it should be equipped with African swine fever virus testing, which was tested negative to ensure that the cleaning and disinfection were qualified. Biosafety measures need to be implemented to ensure the safety of pig farms. To a region and the pig farms within the region, we should make assess the level of biosafety risk periodically, check the gaps.

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A summary of the understanding of classical swine fever

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Introduction

Classical swine fever (CSF) is a highly contagious and economically significant viral disease of pigs. Through three cases, we can understand the clinical symptoms of CSF and provide more experience for the field veterinarians. In the first two cases, infection does not equal to disease, finding irregular edge of the spleen is not necessarily CSF, the infarction of the spleen is not necessarily CSF, and the petechial hemorrhage of the bladder is not always CSF. In the third case, classical swine fever in piglets was confirmed , with thorough diagnostic work-up. To confirm the real causes of the death of piglets, formulate targeted programs and reduce the loss of pig farms.Through three cases, we can understand the clinical symptoms of CSF and provide more experience for the field veterinarians.

Materials and Methods

In case 1, a pig farm in Zhejiang, In the nursery stage, 45 days old piglets are coarse and thin with high mortality.
In case 2, a pig farm in Zhejiang, In the nursery stage, 40 days old piglets are coarse and thin with high mortality.
In case 3, A pig farm in Zhejiang, 10 days after the birth piglets are coarse and thin with high mortality. The diagnosis of the three cases were made by the clinical examination, PCR and histopathology.

Results

In case 1
Field necropsy: Suspect interstitial pneumonia, petechial hemorrhage of the kidney and urinary bladder. PCR: PRRSV positive ,CSFV suspected positive. Histopathology: Confirmed gross suspicion of interstitial pneumonia. Within the alveoli are eosinophilic necrotic debris typical of PRRS. The renal tubules are severely dilated, lined by attenuated epithelium with degeneration; protein and hemoglobin casts are noted; prominent interstitial fibrosis.
In case 2
Field necropsy: finding irregular edge of the spleen, the infarction of the spleen, the petechial hemorrhage of the bladder. PCR: CSFV negative. Histopathology: Slight congestion in the spleen and bleeding at the edge of the spleen, but no atrophy of lymphocytes.
In case 3
Field necropsy: the infarction of the spleen. PCR: CSFV positive. Histopathology: Bleeding and necrosis at the edge of spleen; There is bleeding, fibrosis and calcification in the necrotic area.

Conclusions and Discussion

In case 1, PRRSV PCR positive and significant eosinophilic necrotic debris in alveoli established the diagnosis of PRRS. CSFV PCR suspected positive but lesions typical of CSF are not observered on histopathology. The pigs may be subclinically infected by CSFV but the clinical significance of infection is debatable.
In case 2, CSFV PCR nagative but lesions typical of CSF are not observered on histopathology. The pigs is not infected by CSFV. The edge of the spleen is serrated and may be caused by nonspecific changes during the execution of piglets.
In case 3, in the clinical anatomy, infarction of spleen was seen, positive PCR was detected, and typical pathological changes caused by CSFV were also seen in the pathological tissue sections, classical swine fever in piglets was confirmed.
Infection does not equal to disease, finding irregular edge of the spleen is not necessarily CSF, the infarction of the spleen is not necessarily CSF, and the petechial hemorrhage of the bladder is not always CSF.

Acknowledgments

Hangzhou huitong biotechnology co. LTD in China
Prairie Diagnostic Services (PDS) in Canada

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Fibrocartilaginous embolism in pigs in Brazil

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Introduction

Fibrocartilaginous embolism (FCE) refers to the migration of fibrocartilaginous material of nucleus pulposus through the nearby vasculature, to embolize into the spinal cord vessels most commonly, but also to the lung, brain, vertebrae, and ribs (1). The disease has been described in dogs, cats, horses, turkeys, and pigs (3), although it is usually not considered as a differential diagnosis in clinical cases of neurological disease in swine. The aim of this work is to report the clinical and pathological aspects of cases of fibrocartilaginous embolism in pigs.

Materials and Methods

Four pigs with history of neurologic signs were subjected to euthanasia and necropsy due to the poor prognosis, after attempts of clinical treatment. Additionally, one pig was subjected to necropsy after being found dead without observation of clinical signs. At necropsies, tissue fragments, including spinal cord and brain, were collected, fixed in 10% formalin solution, processed routinely and stained by hematoxylin and eosin (HE) and alcian blue (AB) stains.

Results

Pigs #1 and #2 were gestating sows from group housing systems, with parity 4 and 8, respectively. Pigs #3 to 5 were growing-finishing pigs. Pig #3 was a 175-days-old female; pig #4 was a 100-days-old male; and pig #5 a 170-days-old female. Only pigs #4 and #5 were from the same farm. Pig #1 suddenly presented bilateral hind limb paralysis and was euthanasied 15 days later. Pigs #2 and #3 were found in lateral recumbency and were euthanasied within three days. Pig #4 was found dead without observation of clinical signs. Pig #5 got involved in a fight and some hours later was found in lateral recumbency. On the next day, this pig presented opisthotonus and convulsions, and was euthanasied. On gross examination, in pigs #1, #3 and #4 the spinal cord had focally extensive areas of malacia, characterized as focus of softening of the parenchyma, with yellow or red discoloration. Pig #4 also presented rupture of the urinary bladder associated with uroperitoneum and chemical peritonitis. Pigs #2 and #5 showed no significant changes on gross examination. Microscopically, multifocal or focally extensive areas of necrosis were observed in the spinal cord of pigs #1-4, associated with gliosis, infiltration of gitter cells, and perivascular cuffing of lymphocytes and plasma cells. The lesions affected predominantly the gray matter. In the white matter there was vacuolization and presence of axonal spheroids. The lesion involved the cervical segment in pigs #2 and #3; the lumbar segment in pig #1; and the sacral segment of pig #4. In pig #5 the microscopic findings were restricted to the cerebellum, where there was a mild perivascular inflammatory infiltrate of lymphocytes and plasma cells. In all the cases, a solid, slightly basophilic material, similar to the nucleus pulposus of intervertebral discs, was observed inside arterioles nearby the areas of lesions, especially in the meninges. This material stained in blue in AB stain.

Conclusions and Discussion

The diagnosis of fibrocartilaginous embolism in five pigs was based on the clinical history, necropsy and histological findings. Pig #5 of this report experienced abrupt physical exercise during a fight. All other pigs had no history of managements or fighting, but a fight or trauma cannot be excluded, since these animals were housed in groups. In one report, multiple pigs were concomitantly affected by fibrocartilaginous embolism, and the authors suggested the sorting for slaughter and the genetics for heavily muscled animals as possible predisposing factors (2). The route by which the fibrocartilaginous material travels to reach the spinal (or brain) vessels is not proven, but it is suggested that a trauma to a metaplastic nucleus pulposus causes its fragmentation, and that the pressure of the trauma forces small fragments into the vessels (3). This disease must be considered as a differential diagnosis in cases of neurological disorders in swine, especially in sporadic cases with sudden onset of clinical signs suggestive of spinal cord lesion.

Acknowledgments

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Time-to-stability and time-to-baseline production in three Chilean breed-to-wean farms after load, close and PRRSv live-virus inoculation

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most important viral diseases in pigs. In Chile, PRRS virus was detected in 1999 and eradicated in 2007 through a combination of herd closure and depop/repop; however, a new strain was detected in 2013 (1). In the first and second quarter of 2014, three breed-to-wean farms from the Comercial Maxagro company broke with a type 2 PRRS. The company rapidly made the decision to begin the elimination process. The objective of this field study was to determine the time needed by the farm to return to baseline production and consistently wean PRRSv negative piglets.

Materials and Methods

Between February and April 2014, three breed-to-wean farms from the Comercial Maxagro company in Chile broke with PRRSv for the first time in the history of the company. These naïve farms had 950, 3200 and 4050 sows and were located within 10km from each other. Within the next few days after viral introduction, each herd was loaded with as many gilts as possible and closed. One week after the virus was detected at each farm, RT-PCR positive serum was diluted at a ratio of 4 ml of serum per 1 liter of PBS and each female and heat checking boar were intramuscularly injected with 2 ml of the diluted serum on one day. Approximately sixteen weeks post-inoculation (PI), McRebel procedures such as no cross-fostering, AIAO flow by farrowing room, no stepping into farrowing crates, intensive cleaning and disinfecting and use of one needle per litter were implemented in each herd. Testing began at 20 weeks PI every 2 weeks with a sample size of 30 due-to-wean pigs (one per litter) and once 3 consecutive negative test results were achieved, the sample size was increased to 60 piglets. Sera was tested in pools of 5. Stability was defined as the completing 6 consecutive negative PCR test results. Time-to-baseline production was achieved when the number of due-to-wean piglets reached the farms' established goal before the outbreak.

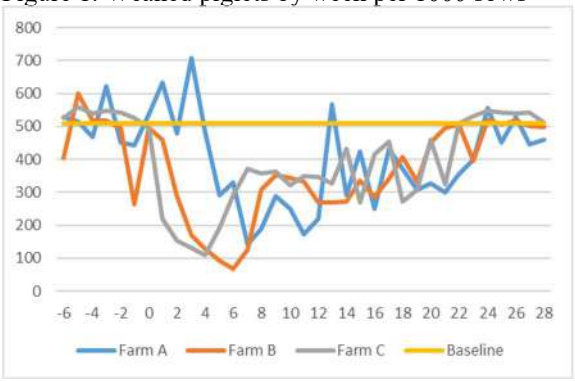
Results

Farms reached stability between 36 and 41 weeks PI whereas time-to-baseline production was achieved between 21 and 23weeks PI. The number of piglets not weaned from the inoculation until the recovery of the usual number of weaned piglets was in average 4,643 piglets per 1,000 sows.

Table 1. Pooled serum PRRSv PCR test results from due-to-wean at weaning through time in 3 breed-to-wean Chilean farms.

Week PI	21	25	27	29	31/32	33/34	35/36	38/39	40/41
Farm A	Neg 0/6	Pos 1/6		Neg 0/6	Neg 0/6	Neg 0/6	Neg 0/12	Neg 0/12	Neg 0/12
Farm B	Pos 3/6	Neg 0/6	Neg 0/6	Neg 0/6	Neg 0/12	Neg 0/12	Neg 0/12		
Farm C	Pos 1/6	Pos 1/6	Pos 1/6	Neg 0/6	Neg 0/6	Neg 0/6	Neg 0/12	Neg 0/12	Neg 0/12

Figure 1. Weaned piglets by week per 1000 sows



Upon stability, all farms introduced replacement gilts. All farms continued to wean PRRSv free pigs.

Conclusions and Discussion

The company was successful in eliminating the PRRSv wild type virus through load, close, live-virus inoculation and farrowing-gestation activities aimed at avoiding within herd transmission. Herd status and productivity were recovered within the range of what has been previously reported (2, 3).

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Characterization of the production losses in three Chilean breed-to-wean farms during a PRRSv outbreak

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most important viral diseases in pigs. Clinical presentation of PRRS includes abortion, premature farrowing, stillbirths, and increased pre-weaning mortality (1) The objective of this study is to characterize the production losses in 3 breed-to-wean PRRSv naïve farms after the introduction of a wild-type virus and through the load-close-expose process to eliminate the virus.

Materials and Methods

Between February and April of 2014, three recently infected Comercial Maxagro breed-to-wean farms began the elimination process of a wild-type PRRSv through the load-close-expose program. Exposure was performed by live-virus inoculation of the recently introduced virus. The study assessed the weekly impact in different performance parameters beginning on the week of the outbreak.

Results

Peak mortality in females and abortions occurred between week 1 and 2 post-inoculation (PI) (Figure 1). Maximum weekly mortality and abortion rate were 0.087% and 12.4%, respectively. Stillborn rate peaked (e.g. 44.6%) between weeks 3 and 4 PI. Mummies increased up to 81.9% 11-14 weeks PI. Pre-weaning mortality increased up to 81.9% between the third and sixth week PI (Figure 2). Born alive decrease was seen in two occasions, the first between 2 and 4 weeks PI and the second between weeks 12 and 16 PI with the minimum being 5.77 piglets born alive per female per week (Figure 3).

Figure 1. Abortion rate by week

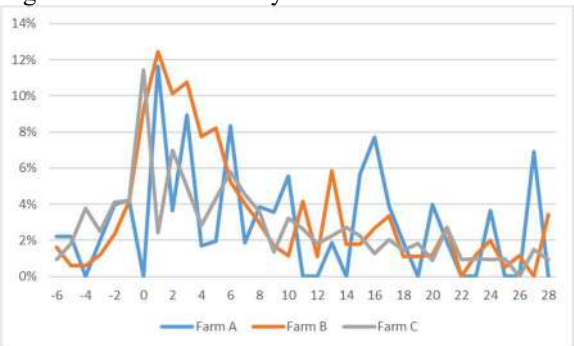


Figure 2. Pre-weaning mortality by week

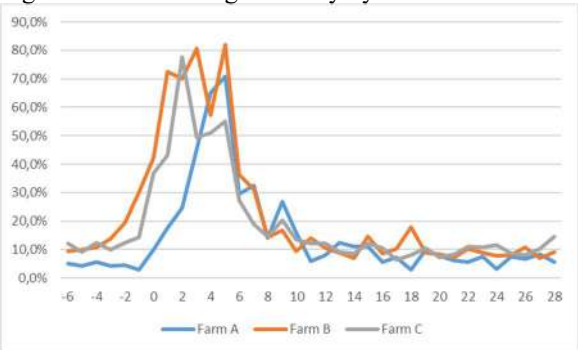
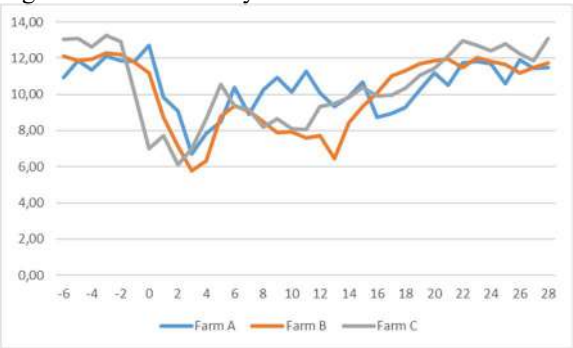


Figure 3. Born Alive by week



Conclusions and Discussion

The peak of the production impact in the different performance parameters assesed correlates with the reproductive stage of the sows at the time of live-virus inoculation.

The first parameter to increase was sow mortality and abortions whereas mummy rate was the last to be affected in those sows that did not abort. Born alive impact was manifested twice, one while mummy rate increased and the second one when stillborn rate increased.

In general, production losses across all three herds were somewhat similar. Interestingly, the magnitude of the impact varied in specific weeks and the cause is not well understood.

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Three vaccine control strategies to control PRDC in a finishing farm in Chile

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Introduction

Porcine respiratory disease complex (PRDC) is a multifactorial syndrome caused by primary, secondary and opportunistic pathogens that affect profitability in pig production (1). In a finishing farm in Chile, PRDC caused mortality rates ranged 4 to 12%. In all the clinical cases, *Actinobacillus pleuropneumoniae* (APP) was identified, isolates were further characterized as serotype 12. Nevertheless, serum samples were mostly negative for ELISA ApxIV. On the other hand, same samples were highly positive for Influenza A ELISA NP, spotting the importance of flu in the clinical case. Then, influenza virus (SIV) was isolated from clinical cases and subtyped as pandemic like H1N1 2009 virus. The aim of this study was to compare 3 vaccine control strategies to control PRDC in this farm, evaluating productive parameters.

Materials and Methods

A total of 3754 pigs were allocated into 3 different treatment groups. The group A receive 1 dose of APP serotype 12 autogenous vaccine and 1 dose of Flusure pandemic®, that containing pH1N109, group B receive 2 doses of Flusure pandemic ® and group C received 2 doses of APP serotype 12 autogenous vaccine. For all protocols, the vaccination was performed in 70 and 90 days-old pigs. All pigs were also vaccinated with PCV2 and M hyo vaccine at the nursery.

Productive parameters by group, as mortality rates, feed conversion rate and daily weight gains (ADG) were compared. Using 200 pigs per group individual carcass weight and pulmonary score were determinate at the slaughterhouse using the Piffer and Brito model (2). Statistical analyses were performed using ANOVA model followed by Tukey-Kramer analysis.

Results

Average performance parameters by group are shown in Table 1. ADG was similar in groups B and C, the mortality rate was low in all groups.

Table 1: Average performance parameters

	Group A	Group B	Group C
n	1249	1256	1249
Initial weight	30,89	29,64	31,39
Initial age	77,0	75,5	79,5
Final weight	125,42	128,27	127,15
Final age	175,1	173,7	175,7
ADG	0,964	1,004	0,995
Mortality rate	1,60	1,59	0,88
Feed conversion	2,701	2,694	2,641

Individual carcass weight and pulmonary score are shown in Table 2. Group B and C had significant statistical differences to group A in carcass weights. The mean of lung injurie was lower in group B, but no significant differences were found between groups. Additionally, the prevalence of pneumonia and the pneumonic index was lower in group B.

Table 2: Carcass weight and pulmonary score

Group	Mean carcass weight (kg)	Mean (%) of lung injurie	Prevalence of pneumonia	Pneumonic Index of the herd
A	102.5[a]	4.02[a]	58.33%	0.694
B	106.6[b]	2.98[a]	40.28%	0.505
C	106.5[b]	3.97[a]	50.23%	0.707

Conclusions and Discussion

First, the three groups presented lower mortalities rates compare with previous batched without treatment. Interesting, the lower mortality rate was observed in group C, two doses of APP vaccine may protect against a more serious presentation of the disease, which is always related pigs mortality in finishing pigs.

Carcass weights were higher in groups B and C, using 2 doses of a vaccine administered three weeks apart (following manufacturers recommendations) than using one dose of each vaccine in group A. Although there are no significant statistical differences between the carcasses weight and mean of lung injurie of groups B and C, the carcass weight of the pigs of group B was achieved 2 days before. This could be related to the fact that this group had a lower prevalence of pneumonia and pneumonia index. Which suggest that SIV was presented as primary pathogen and APP was presented as secondary infection of SIV. Other studies revealed that SIV increased lung lesions and facilitated secondary infections in the porcine lung (1).

Both SIV and APP are important pathogenic co-infections in this farm that must be controlled. The results demonstrated that clinical presentation can be controlled more efficiently using double dose of APP or SIV independently. However, double dose of SIV seems to be more useful reducing in prevalence and pneumonic index.

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Intervention with Ingelvac CircoFLEX® in a Dutch closed herd improved finishing pig performance

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Introduction

Circo virus vaccination is adapted broadly in the swine industry. However, some farms still do not implement vaccination against this disease, especially when typical symptoms are lacking. The objective of this study was to identify the reason for suboptimal ADG in a non PCV2 vaccinating farm and to evaluate the implementation of Ingelvac CircoFLEX® on zootechnical parameters like ADG.

Materials and methods

In the summer of 2017 a cross sectional serological profile was made in a 600 head farrow to finish herd. The ADWG (Average daily weight gain) was much below the Dutch average (783 gram vs. 810 gram). At the age of 10, 16 and 22 weeks 10 blood samples were taken. Serum was analyzed using ELISA for the determination of antibodies against PRRS, PCV2, SIV, Mhyo and Lawsonia. Additional 3 oral fluid samples were taken per age group. Combined infections with PCV2 and PRRS were found during the finishing pigs phase starting between the age of 10 and 16 weeks (Table 1 and

Table *2*).

Table 1 Positive antibody testing results (%) of the different age groups (ELISA).

Age (n)	PRRS	PCV2	SIV	Mhyo
10 (10)	40	20	20	100
16 (10)	90	0	0	10
22 (10)	100	100	0	30

Table 2 Positive oral fluid testing results (%) of the different age groups (PCR).

Age (n)	PRRS	PCV2	SIV	Mhyo
10 (3)	0	0	0	0
16 (3)	0	33,3	0	0
22 (3)	0	0	0	0

It was decided to vaccinate the pigs with Ingelvac CircoFLEX® in the farrowing house from the start of 2018. A historical comparison was made based on production data from the management system. To provide reliable data, ADG and FCR were calculated for a period 4 months (a full barn turnover period). These calculations were made for each and every subsequent month from the beginning of 2016 until the end of 2018. Figures adjusted for starting weight differences were used (Agrovision; standardized for growth 25-117 kg live weight).

Results

ADG improved with 53 grams a day between 2017 and 2018 (Table 3 and Figure 1), which is more than on genetic progress could be expected. FCR improved with 0,03 kg.

Table 3 Historical performance data of 2017 and 2018.

year	FCR (kg)	FCR (energy value)	Mortality	ADWG
2017	2.47	2.73	1.3%	799
2018	2.43	2.68	2.5%	852

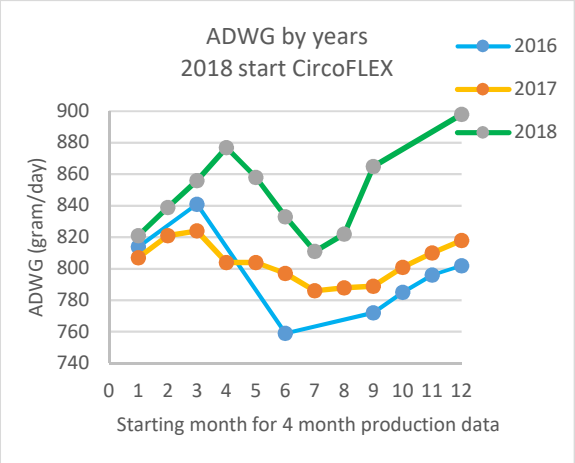

Figure 1 Average daily weight gain from 2016 (blue) 2017 (orange) and 2018. The hot European summer of 2018 reduced the ADG, but still higher in comparison to previous years.

Discussion/Conclusion

Mortality increased for 2018 from 1.3 to 2,5 %, which is slightly above the Dutch average of 2.3%. This increase in mortality was due to a lowed vitamin E status due to a lower availability of vitamin E in 2018. With the current gross margin per finishing pigs of €60,- an improvement in ADG of 53 grams (799 to 852 grams) results in a financial benefit of €60/799*53 = €3.98 per finishing pig. FCR reduction resulted in an additional feed saving of 5 kg per pig, which is an extra saving of €1.25 per pig. In total, intervention with Ingelvac CircoFLEX® increased zootechnical performance with an economic value of €5.23 per pig.

Changes in breeding gilts acclimation resulted in herd stability for *Mycoplasma hyopneumonia* with reduced lung leasions in the finishing pigs

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Introduction

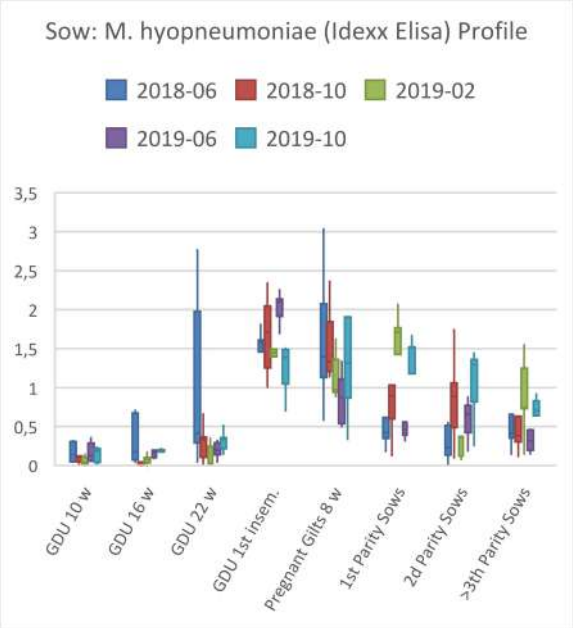
Acclimation strategies of breeding herd replacement stock is important in the prevention of the spreading of infections to offspring, In particular in the case of Mycoplasma hyopneumoniae (Mhyo), prolonged shedding from breeding stock to offspring can occur (1) when acclimation practices are not well managed, leading to increased exposure of the offspring (2).

Materials and methods

On a 6000 head sow farm problems occurred with early and wide spread mycoplasma infections in the summer of 2018. Antibody testing of breeding stock showed irregular spread of mhyo antibodies at the end of the gilt developer phase and subsequent periods during first gestation and first lactation. Gilt acclimation practices were evaluated and major changes were made to improve this. Objective of the acclimatization was to obtain a homologous mhyo status of the breeding gilts, resulting in less infections of the finishing pigs. Gilts in the gilt developing unit (GDU) were acclimatized to the breeding sows before insemination. At the age of 18 weeks the gilts were exposed to oral fluid ropes which were pre-exposed to the gestating sows. At an age of 20 weeks, one gilt per pen in the GDU was introduced to the just weaned sows by group housing them in a large room for several hours with adequate time for direct exposure and contact with the sow herd. Breeding gilts we revaccinated against mycoplasma three weeks before introduction to the breeding sow herd. Effect of these changes were monitored by repeated serological surveillance of breeding stock. Every 4 months 5 blood samples were taken of the following age groups: GDU: 10, 16, 22 weeks; insemination age, mid gestation and at lactation of first, second and >third parity. Blood samples were tested with Elisa for Mhyo (Idexx Mhyo Ab test). A year after implementation a slaughterhouse check was performed to assess the presentence of Mhyo like lesions.

Results

Serological status changed over time after implementation of the acclimation strategy. Gilts showed a homologous distribution of antibodies presence at insemination. After the acclimation period at the end of the GDU, Mhyo antibody distribution became more homologous with less variation.



Serology of off spring showed a clear reduction in the amount of positive animals at the age of 22 weeks, which is a indication for lower prevalence of Mhyo colonization. Lung health of the offspring was evaluated using a routine slaughter house check.

Discussion

The changes in the gilt acclimatization resulted in less variance in gilt serological profiling. The synchronization of natural mhyo infections before insemination, combined with vaccination of the gilts resulted in less colonization of the offspring at 22 weeks of age in combination of a very low presence of lung leasions at slaughter.

Conclusion

This study gives an example of a pragmatically and successful program in controlling enzootic pneumonia in a large closed farrow to finish system.

References:

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Systemic bacterial embolism in growing-finishing pigs

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Introduction

Tail lesions from cannibalism are often seen in swine herds, especially in growing-finishing pigs. Many of these animals develop suppurative and abscessed multifocal lesions of hematogenic origin, secondary to the lesions of the tail. Cannibalism is also a concern in pig production due to the condemnation of carcasses affected by secondary embolic lesions (Braga et al. 2006, Kritas & Morrison 2007, Marques et al. 2012,). The aim of this work is to report deaths of growing-finishing pigs due to systemic bacterial embolism, based on the necropsy examination, histopathology and bacteriology, and highlight the association between tail biting and bacterial embolism in pigs.

Materials and Methods

During a study on the causes of death and reasons for euthanasia of growing-finishing pigs, necropsies were performed in pigs that died spontaneously or were euthanasied in two farms in Santa Catarina State, Brazil, in a period between 2018 and 2019. At the necropsy, fragments of organs were collected, fixed in 10% formalin solution, processed routinely to histopathology. Fragments of organs and swabs from suppurative lesions were subjected to bacterial isolation, seeded on Blood and MacConkey Agar plates and incubated at 36 ° C +/- 1°C for 24-48 hours in an aerobic atmosphere. Pigs included in this work had three or more abscedative and suppurative lesions, with or without primary lesions at the necropsy.

Results

Thirty-three cases of systemic bacterial embolism, out of a total of 610 necropsies performed (5.4% - 33/601), were diagnosed in this study. The disease affected pigs aged between 120 and 190 days (median 180 days), with a predominance of males (72.72% - 24/33). The primary lesions, which acted as the primary focus of infection for posterior hematogenous dissemination of bacteria, were identified in 75.7% of the cases (25/33); 92% of these corresponded to tail biting lesions (23/25) and 8% to cases of pododermatitis (2/25). The clinical and pathological picture was characterized by poor growth, dyspnea, enlarged joints, frequently leading to paresis or paralysis of pelvic limbs. The combinations of suppurative and abscessed lesions, mainly arthritis (25/33), embolic pneumonia (21/33), and osteomyelitis, especially in the vertebral column (12/33), were observed. Less frequent lesions included endocarditis (2/33), splenitis (2/33), lymphadenitis and serositis (1/33 each). Bacterial isolation was possible in 36.36% of the cases (12/33), with isolation of *Trueperella pyogenes* and *Pasteurella multocida* type D in 12% of the cases (4/33 each), *Staphylococcus* sp. in 9.09% (3/33) and *Streptococcus suis* in 3.03% (1/33).

Conclusions and Discussion

Systemic bacterial embolisms are caused by secondary and opportunistic bacterial infections, which eventually spread by hematogenic pathway from a primary lesion and can cause suppurative lesions and abscesses. The main association with a primary injury in this study was laceration of the tail due to biting, as observed by other authors in pigs with embolic lesions during the slaughter process (Braga et al. 2006, Marques et al. 2012). There was also a predominance of male pigs affected by this condition, which may indicate a predisposition to caudophagy, as observed by other authors (Walter & Bilkei 2006, Kritas & Morrison 2007). Other factors that can predispose to this condition are related to stress, such as overcrowding, competition for food, nutritional factors, humidity, temperature, and gas levels (CO_2 or NH_3). In addition to its importance as a cause of carcass condemnation in the slaughterhouse, the present study highlights the group of embolic diseases as an important cause of death of growing-finishing pigs associated with tail lesions in Southern Brazil.

Acknowledgments

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Sudden death associated with liver lobe torsion in pigs

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Introduction

Partial or complete liver lobe torsions have been reported in pigs, dogs, cats and horses (2). Researches report the alterations in the position of the abdominal organs as important causes of death of sows, being the gastric and splenic torsions the most commonly diagnosed, while the liver torsion is less frequent (1,5,6). In pigs, torsions commonly occur in the left lateral lobe, for being the largest lobe in this species, as well as being connected to the left medial lobe by a narrow band of tissue (2,4,7). Thus, the aim of the present study was to describe 13 cases of liver lobe torsion in pigs.

Materials and Methods

Technical visits were carried out in four pig farms, located in the Brazilian states of Santa Catarina, Mato Grosso and Paraná. Three farms were of sows and the fourth farm was a growing and finishing pig operation. During the visits, necropsies were performed on pigs that died spontaneously. Information regarding the clinical history, reproductive stage and age of the pigs was recorded. At necropsy, fragments of tissue were collected and fixed in a solution of formalin 10% for histopathology.

Results

We observed 13 cases of hepatic torsion in pigs, nine died suddenly and four pigs presented hyporexia, apathy and vomiting just hours before death. Of the 13 cases, nine were sows (eight in lactation and one weaning-to-estrus interval - WEI); two were unmated gilts; and two were finishing pigs (average 175 days of life). At necropsy, hepatic lobe torsion was observed, usually with rupture and hemoperitoneum. In all cases, the left lateral lobe was twisted, and the torsions ranged from 360° to 720°. Grossly, the twisted lobe was swollen, dark red, heavy, firm and with fissures on the capsular surface associated with fibrin deposition; or it was mild due to emphysema and loss of parenchyma after capsular rupture. Microscopically, there was massive necrosis of hepatocytes associated with intense hemorrhage and fibrin deposition. Sinusoid dilatation and atrophy of hepatocyte cords was also observed. Some air spaces (emphysema) was observed in the twisted lobe associated with these lesions. In the adjacent parenchyma there were marked congestion and hemorrhage, and in the Glisson's capsule, moderate deposition of fibrin and neutrophilic inflammatory infiltrate were also evidenced.

Conclusions and Discussion

Liver torsion is an important condition to be considered as a cause of sudden death in pigs, especially in lactating sows. The most intense handling in this phase may be related to torsion, as the sows are more agitated, lie down and get up several times a day to breastfeed and feed (4). The affected pigs die of hypovolemic shock, due to rupture of the liver and consequent hemoperitoneum. The left lateral lobe is the most affected due to its anatomical peculiarities in swine. As a differential diagnosis for sudden death in pigs, necrotic hepatitis by *Clostridium novyi* should be considered (3). The history and anatomopathological findings in these cases were sufficient for the diagnosis of hepatic lobe torsion. Studies describing liver torsions in pigs are scarce and old, justifying the importance of this report.

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Report of PCV-2 infection causing reproductive failure and improvement after revaccination with Ingelvac CircoFLEX® in Malaysia

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Introduction

Porcine circovirus type 2 (PCV2) has a great impact on breeding herds, predominantly associated with increased stillborn and mummified fetuses at parturition. (1, 2) Reports have shown that mass vaccination of sows against PCV2 would improve the reproductive performance and are proven to be safe. This study reports a recurrence of PCV2 infection in the sow herd after discontinuation of mass vaccination against PCV2.

Materials and Methods

This case report is a single site farm in Malaysia, with 800 sows. The farm experienced an increase in the percentage of mummified fetuses from sows of different parities from November 2017 until March 2018. In March 2018, the mummified fetuses of these sows tested positive for PCV2 by using Real-Time PCR. Mass vaccination of Ingelvac CircoFLEX® (Boehringer Ingelheim Vetmedica GmbH) was done in sows in April & May 2018 and there has been a decreased of mummified fetus from 2.69% to 1.54%. A booster of the whole sow herd was recommended every 6 months. However, the farmer did not revaccinate the sow after 6 months. In February 2019, the farm experienced an increase in the percentage of mummified fetuses consecutively for 3 months. Following these findings, the sows were again mass vaccinated with Ingelvac CircoFLEX® in April 2019. Reproductive parameters were evaluated for the period before (October 2018-April 2019) and the period after implementation of sow vaccination (May 2019-October 2019). The statistical analysis was generated using Minitab software version 17.

Results

During the first outbreak, a reduction of the percentage of mummified fetuses was seen from 2.69% (outbreak) to 1.54% (after Ingelvac CircoFLEX®). A recurrence was seen after October 2018, when a booster vaccination was not done in the farm. After revaccination using Ingelvac CircoFLEX® in April 2019, reduction of the percentage of mummified fetuses was seen from 1.83% to 1.36%. (Figure 1)

The results are summarized in figure 1.

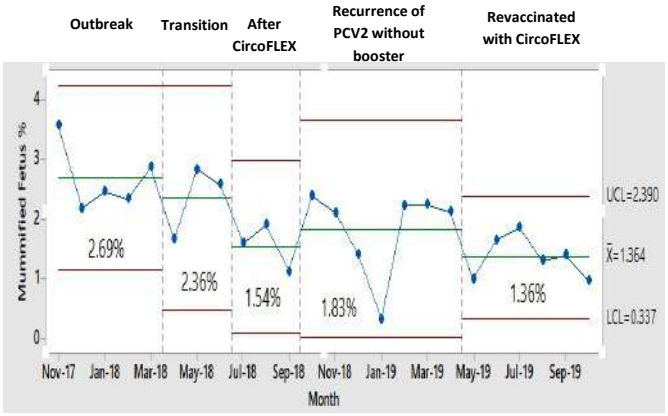

Figure 1. Monthly percentage of mummified fetus.

Discussion and Conclusion

In this case, PCV2 antigen was detected by Real-Time PCR from the heart and lymph nodes of the mummified fetus. The results of this field observation further confirm that Ingelvac CircoFLEX® vaccination is an effective tool to improve the reproductive performance of the breeding herd when there is a decreased in the percentage of mummified fetus after vaccination. Besides that, the study shows that there is an obvious increased in the percentage of mummified fetus when a booster was not done in the farm. Therefore, it is important to do a booster of Ingelvac CircoFLEX® every 6 months to reduce/prevent reproductive failure associated with PCV2 infection.

Acknowledgements

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A report of an increased mortality in breeding sows due to *Clostridium novyi* infection

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Introduction

Clostridium novyi is a large, strict anaerobic and gram-positive rod. It can be differentiated in A, B, C and D by the production of toxins α and β (5). Only types A and B were reported to affected pigs and it is found the soil, gut and faeces (5). Pathogenesis of *C. novyi* infection was categorized as histotoxic. Gross lesions include distention of the carcass, purple discoloration of the skin, generalized edema and subcutaneous infiltration with bubbles fluid in pericardial, pleural, and abdominal cavities All organs, in particular the liver are softened, spongy, gas-filled, and necrotized often referred to as "aerochocolat liver" (5). The disease seems to have the highest incidence in breeder's sows, in the peripartum period. In general, sows seem to have a good physical condition and suddenly die, with a fast and unusual cadaveric decomposition (2). Clostridia are difficult bacteria to grow, being *C. novyi* the most complex. In addition, the simple isolation of clostridia species does not confirm the diagnosis due to the rapid postmortem invasion and proliferation *C. novyi* as well as the toxin detection in healthy pigs (5) The use of PCR procedures can allow rapid identification of toxigenic type *C. novyi* involved (3). We report the procedures carried-out for the diagnoses of *C. novyi* infection associated with an increased mortality in breeding sows.

Materials and Methods

Since 2017 a multisite pig herd of 8850 sows, reported an increased rate mortality of the breeder's stock (min 7.2%, max 8.4%) that exceeded the standard rate of the genetic line (6%). A non-probabilistic sampling was performed in 5 sows with sudden death, with great post-mortem distention and rapid degree of decomposition for the diagnosis of *C. noyvi*. During the necropsy samples of the liver, spleen and lung were extracted by means of FTA® cards, 2 each organ and sow. The methodology used for the diagnosis was the real time PCR (rtPCR). The extraction, purification by chemical-physical processing was performed by an automated extraction system (QIAGEN). Subsequent DNA amplification of toxins α and β genes of *C. novyi* was performed. Results were expressed semi-quantitatively as low, moderate or large amount of *C. novyi* genetic material.

Results

All the livers examined had the "aerochocolat" aspects. In table 1 the results of the rtPCR are expressed. All the sows presented at least one organ with moderate to abundant amount of genetic material.

Discussion and Conclusion

Clostridium novyi infection has been associated with sudden death with unusually rapid postmortem decomposition (5). The infection affected fattening pigs and old parity sows in the peripartum period. In the study, the sudden death sows were 1 or more parity. Both *C.novyi* type A and B can produce the signs and gross lesions such a necrotic hepatitis. We can conclude that sudden death in the postmortem examined in sows can be caused by *C. novyi* type A because only α toxins was detected. However, the number of studied sows was not representative of all the breeding population. The use of the rtPCR test at the time of necropsy allowed the diagnosis of *C. novyi* infection.

Table 1. Results of the rt-PCR for the detection of α toxins of *C novyi.* alpha toxin.

ID sow	Result	sample
1	POS (+++)	lung
1	NEG	liver
2	POS (++)	spleen
2	NEG	lung
3	POS (+++)	liver
4	POS (+++)	lung
4	POS (+++)	spleen
4	POS (+++)	liver
5	POS (+++)	liver

NEG: not detected, POS (+): low quantity, POS (++): mderate quantity POS (+++): large amount of *C. novyi* genetic material.

Acknowledgments

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Reduction of the mortality rate using a *Clostridium novyi* vaccine

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Introduction

Clostridium novyi is an anaerobic, spore-forming, gram-positive rod and ubiquitous bacteria in swine farms [1,2]. Beyond that, *Clostridium novyi* alpha toxin is the responsible of porcine infectious necrotic hepatitis and sudden death in sows, especially during the late states of gestation and the peripartum[3]. Its diagnosis consists in a rigorous *postmortem* inspection followed by a collection of the liver parenchyma for a subsequent molecular diagnosis using a PCR test. The aim of this study was to evaluate a commercial subunit vaccine containing the α toxin of *Clostridium novyi* as a sudden death prevention tool.

Materials and methods

This study was conducted in a farrow to finish French farm of 360 sows after reporting an increase of the incidence of the mortality rate during 2018, increasing up to 11.6%. The mortality occurred without any specific clinical signs. Systematic necropsies were performed, and the most relevant findings were swelling of the abdomen and degeneration, emphysema and brown colouring of the liver[4]. To confirm the diagnosis, a PCR was carried out and the presence of the gene encoding the α toxin of *Clostridium novyi* type B was detected. The whole farm was vaccinated with SUISENG® following the manufacturer's instructions. To evaluate the vaccine's efficacy, the sow mortality rate was compared between two different periods being Period A from January 2018 until January 2019, when no *Clostridium novyi* vaccines where used at the farm, and Period B ranged from February 2019 until December 2019, when the farm started the vaccination with SUISENG®. Finally, a Wilcoxon test was performed to compare the two periods.

Results

As it is shown, after the new vaccination protocol established, the mortality rate decreased in 2019 from 4 (SD ± 2.3) to 2.2 (SD± 1.6) sows per month (p-value < 0,05) (Fig.1).

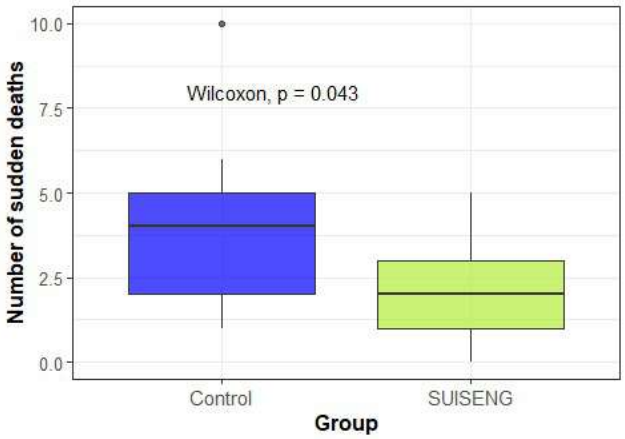

Fig.1: Comparison of the average number of sow mortality rate of period A (blue) and period B (green).

Conclusions and Discussion

Sow mortality is a growing concern on pig farms. There are multiple causes behind it, such as C. *novyi*. It is remarkable to highlight the importance of having a good diagnosis of Clostridia disease in order to identify the mortality source. These results demonstrate the importance of the implementation of a vaccination program against *Clostridium novyi* as a primary measure to reduce the incidence of sudden death in commercial farms.

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Evaluation of the effect of vaccination PRRS MLV vaccine in infected by PRRSv on the grower-finisher pigs

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Introduction

PRRSv remains a very costly disease in the pig industry, especially in grower finisher pigs[1].Vaccination has been demonstrated to be cost effective to reduce clinical sign and mortality of PRRS infection[2]. Despite the application of Type I-PRRS MLV vaccination, there occurred introduction of new type II-PRRSv field infections in a grower-finisher farm. To minimize production loss, Ingelvac PRRS® MLV has demonstrated efficacy against heterologous PRRSv challenge when pigs have already been exposed to PRRSv type II in the field[3]. The objective of this observation was to evaluate the effect of vaccination by Ingelvac PRRS®MLV on previously exposed grower-finisher pigs.

Materials and Methods

A 10,000 head grower-finisher farm received 9-10 weeks old pigs from 2 different sow farms. All pigs were previously vaccinated by Type I MLV vaccine at 14 days old at the sow farms. After moving to the grower-finisher farm, pigs started showing distress followed by respiratory sign which resulted in high medication cost, high mortality and wasting. Diagnosis was made by PCR testing for PRRSv infection throughout age group from suckling to grower pigs (n=10 in each group of age)(table 1). The pigs ranging from ages 9-15 weeks old were vaccinated with Ingelvac PRRS®MLV (6 houses with 4,338 pigs) in order to reduce clinical signs and losses while at the grower-finisher farms. Pigs age 16 weeks to market (6 house with 4,256 pigs) were not vaccinated but maintained strict biosecurity and people flow. To evaluate the effect of vaccination, mortality weeks were compared between the groups (vaccinated vs non vaccinated) by Chi square test, OpenEpi Version3.

Results and Discussion

Vaccinated pigs had significantly less mortality compared to the non-vaccinated pigs. The feed intake trend during the first 6 weeks is shown in Figure1. The infected-vaccinated pigs had an advantage in feed intake compared to the non-vaccinated pigs.

Table1. PCR results from PRRS testing

	Stillborn	2-3 wks	5-6 wks	7-8 wks	10-11 wks
Sow source 1	Type II	TypeII	TypeI&II	TypeI& II	Type II
Sow source 2	Type II	Type I	Type II	TypeII	Type II

Table 2. Mortality and feed intake between vaccinated VS non-vaccinated group

	Infected Nx	Infected Vx	P-value
No. Housing	6	6	
Pig in	4,256	4,338	
Pig dead	174	86	
% Mortality	4.08	1.90	<0.05

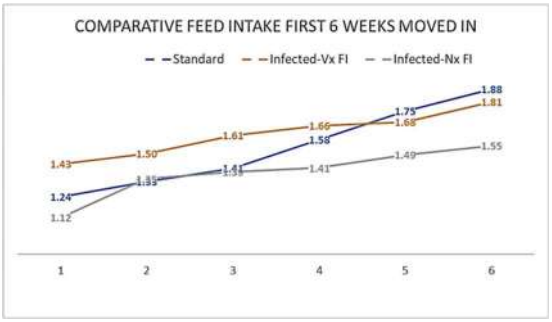

Figure1. The comparative Feed Intake during first 6 weeks after entering grower-finisher farm

Discussion

Infection from PRRSv type2 from the field virus in grower-finisher farms is common in high density pig areas. To minimize losses, agile diagnostics and intervening actions are the keys. The combination of immunization according to a farm's epidemiology and pig-people flow management can help to reduce the impact of PRRS on the performance as well as the circulation in a production system.

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Eosinophilic granulomatous myocarditis in growing-finishing pigs

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Introduction

A number of bacterial and viral etiologies can induce myocarditis in swine, including septicemia, Encephalomyocarditis virus, Foot-and-mouth disease virus, porcine circovirus 2, PRRS virus, Aujeszky's virus, Swine vesicular disease virus (1) and porcine parvovirus (2). Less commonly, parasitic stages of *Toxoplasma gondii*, *Trichinella spiralis*, *Taenia solium* and *T. saginata* may infect the myocardium and produce nodular or cystic lesions. Pigs with myocarditis may have altered cardiovascular function, induced by necrosis and degeneration of cardiomyocytes. These factors may result in acute death associated with dysrhythmia or contribute to chronic heart failure (1). The aim of the present study was to investigate two cases of atypical eosinophilic granulomatous myocarditis in growing-finishing pigs.

Materials and Methods

Clinical information was obtained directly from the field veterinarians and swine farm owners during on-site visits. Two growing-finishing pigs from two farms in Santa Catarina state, Brazil, aged 160 and 135 days, presented with history of sudden death. Necropsies were conducted, and gross lesions were recorded. Tissue sections were fixed in 10% buffered formalin, paraffin embedded and stained with hematoxylin and eosin (HE). Fresh heart samples were submitted for bacterial culture on Blood and MacConkey Agar plates. In addition, sections of the heart were stained with periodic acid-Schiff (PAS). Immunohistochemistry (IHC) for porcine circovirus type 2 (PCV2) was performed on heart and lymph node sections. *Toxoplasma* sp. was tested by performing IHC on heart fragments. *In situ* hybridization (ISH) for PCV2 and porcine circovirus type 3 (PCV3) was performed in sections of the heart.

Results

Both cases occurred in the month of November 2018 in two farms GRSC (Certified Suidae Breeder Farms). The first farm had 20.000 pigs and the second farm had 18.000 pigs. The first case was a 160-day-old female, from the first farm, which had been selected for breeding. The gilt died while being moved from one facility to another. The second case was a 135-day-old female, which was found dead in the stall. Previous clinical signs were not observed. At necropsy, both gilts present similar lesions and gross findings were characterized by a markedly enlarged heart. The myocardium was diffusely firm, presented extensive white areas interspersed with white, multifocal to coalescing, soft nodules, measuring 1-2 mm in diameter, which also extended to the pericardium and endocardium, usually adjacent to blood vessels. Marked hydropericardium was also observed in both cases. The lungs were diffusely red and the oozed serosanguinous fluid at the cut surface. The liver presented marked enlargement and evidenced lobular pattern (nutmeg liver). Microscopic findings observed in the heart were characterized by inflammatory infiltrate composed predominantly of eosinophils. Small clusters composed of epithelioid macrophages, multinucleated giant cells, lymphocytes and plasmocytes were often observed, sometimes forming nodules. The inflammation extended to the pericardium, which showed fibrous connective tissue proliferation and neovascularization. Associated with the inflammatory infiltrate, there was multifocal necrosis of the cardiomyocytes. The liver had moderate diffuse congestion. No bacterial growth was observed in the cultured heart samples. The IHC and ISH were negative for the pathogens tested. No microscopic findings compatible with a parasitic disease were observed.

Conclusions and Discussion

The possible etiologic agent involved in these cases of myocarditis in the affected pigs remains unknown. The infectious agents most frequently detected in the swine population in Brazil were tested and yielded negative results. These results emphasize the importance of future studies involving the search for unusual agents in Brazilian pig herds. Similar to eosinophilic myocarditis reported in humans, the described condition is a life-threatening acute inflammatory heart disease. Clinical cases are generally isolated and associated conditions are identified in approximately 65% of patients (3).

Acknowledgments

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Exploring mortality rates and cause of death in high health boar studs in Latin America

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Introduction

Boar studs contribute importantly to the genetic improvement and production performance of the contemporary swine industry. High health boar studs reduce the risk of transmission of viral diseases through semen. The biosecurity standards, infectious disease surveillance, nutrition requirements, production management, and semen quality of these production sites are well studied. However, the mortality rates and main causes of death are not completely understood. Understanding mortality rates and cause of death in high health boar studs is important to design better health intervention to improve animal health and optimize boar performance. Therefore, the objective of this study was to explore the main cause of death and distribution of mortality rates among 10 high health boar studs in Latin America during 2019.

Materials and Methods

The cause of death and 2019 mortality rate of ten high health boar studs in three countries of Latin America Mexico (3), Colombia (2), and Brazil (5) were analyzed. The health status for each boar stud was established based on the epidemiological surveillance and current laboratory diagnosis. The 2019 mortality rate was estimated based on the number of dead animals divided by the average inventory during the year. The cause of dead was classified as infectious (e.g polyserositis, pneumonia, peritonitis, endocarditis, septicemia, bladder infection), not infectious (e.g gastric ulcer, intestinal torsion), sudden death, and euthanasia (in compliance with animal well-being policies). Tabular methods were used to compare the cause of death by boar stud, month and country and considered significantly different if the p value of the Chi square test was lower than 0.05.

Results

During 2019 all boar studs were negative to porcine reproductive and respiratory syndrome virus (PRRSv), *Actinobacillus pleuropneumoniae* (APP), and porcine coronavirus (Porcine epidemic diarrhea (PED), transmissible gastroenteritis (TGE) and porcine delta coronavirus (PDCoV)). Moreover, three boar studs were positive to *Mycoplasma hyopneumoniae* (Mhp. The total number of boars in Brazil (BRA), Colombia (COL) and Mexico (MEX) were 2114, 312 and 960 respectively for a total of 3386 boars. The overall 2019 mortality rate was 9.57% (324 out of 3386), ranged between 3.31% and 12.1% (Table 1) and euthanasia accounted for 2.4 % (81 out of 3386) of the mortality. Additionally, boar stud No 5 had a significantly lower mortality rate (3.3%) than all other boar studs ($p<0.05$). However, the mortality rate between the remaining nine boar studs was not statistically different ($p=0.09$) and ranged between 4.88% and 12.1%.

Table 1. 2019 mortality rate in 10 high health boar studs in Brazil, Colombia, and Mexico.

Boar stud	Country	No. of deaths	Inventory	% Mt	Mhyo
1	BRA	17	150	11.33	-
2	BRA	38	511	7.44	-
3	BRA	51	448	11.38	-
4	BRA	90	854	10.54	-
5	BRA	5	151	3.31	+
6	COL	6	123	4.88	+
7	COL	22	189	11.64	+
8	MEX	48	419	11.46	-
9	MEX	32	417	7.67	-
10	MEX	15	124	12.10	-
Total		324	3386	9.57	NA

Overall, the cause of death of 324 boars during 2019 was attributed to sudden death (33%), infection (23.1%), euthanasia (25%), and not infectious (18.8%) causes. The percent of euthanasia illustrates compliance with animal well-being policies. The main cause of death was statistically different between countries (Table 2, $p<0.001$) with higher number of sudden deaths in BRA (36.8%), higher number of infectious causes in COL (39.3), and higher number of euthanasia in MEX. This difference suggests misclassification of cause for sudden death or euthanasia in some cases. Finally, the main infectious cause of death was pneumonia (64%, 48 out of 75) and septicemia (9%, 7 out of 75).

Table 2. Distribution of dead animals during 2019 by cause and country in 10 high health boar studs in Brazil, Colombia, and Mexico.

	Infectious	Not infectious	Euthanasia	Sudden death	Total
BRA	43 (21.4)	47 (23.4)	37 (18.4)	74 (36.8)	201 (100)
COL	11 (39.3)	5 (17.9)	7 (25.0)	5 (17.9)	28 (100)
MEX	21 (22.1)	9 (9.5)	37 (38.9)	28 (29.5)	95 (100)
Total	75 (23.1)	61 (18.8)	81 (25.0)	107 (33.0)	324 (100)

Conclusions and Discussion

Understanding the cause of death in high health boar studs is crucial to improve animal health and performance. In this study, complying with animal well-being (euthanasia) accounted for 2.4% of the mortality. Establishing an accurate cause of death is not easy because fresh tissues for histopathology or bacteriology are not always available. Future studies require a better differentiation of the cause of sudden death. Infectious cause of death was more likely related to endemic pathogens (e.g. *Haemophillus parasuis*, or *Streptococcus suis)* and acclimation programs may reduce their impact on mortality rate.

Detection of Senecavirus A and IgG antibodies over time after an outbreak in a breeding herd: A Case Report

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Introduction

Senecavirus A (SVA) is a member of the Picornaviridae family and is associated with vesicular disease in pigs[1]. This virus has been responsible for the constant increase in foreign animal disease (FAD) investigations in the United States. Out of the 2,072 FAD investigations in 2018, 1,592 were reports of vesicular lesions in pigs[2]. Currently there is no data related to the duration of detectable levels of IgG in serum and virus shedding in processing and oral fluids after an outbreak in breeding herds. The objectives of this study are to determine the duration of detection of SVA RNA in processing and oral fluids, and IgG antibodies in serum over time after an outbreak in a breeding herd.

Materials and Methods

A 6,000 commercial farrow-to-wean herd located in the Midwestern United States undergoing an SVA outbreak was conveniently selected for this study. No attempt of massive SVA exposure or SVA elimination was done after the outbreak. A cohort of 60 sows across parities was randomly selected from one breeding group and then followed through monthly blood sampling. Processing fluid testing comprised of weekly testing from three weeks before until 26 weeks after the outbreak. Oral fluids from on-site gilts (growing and finishing stages) were sampled weekly or bi-weekly after the third week of the outbreak. Blood samples were serologically tested for IgG presence by an indirect immunofluorescence assay (IFA). Processing and oral fluids were SVA tested by qPCR at the University of Minnesota Veterinary Diagnostic Laboratory.

Results

Fifty-nine out of the 60 sows tested positive at least once throughout the study (Table 1). There is titer variability within sows through time. Processing fluids qPCR results showed a clear Ct decreasing trend followed by an increasing trend through time (Figure 1). Oral fluids from replacement gilts at growing and finishing phases tested positive by qPCR at days 23 and 47 after the outbreak, with mean Ct values of 27 and 35 respectively.

Conclusions and Discussion

SVA detectable antibodies last at least 7 months with high titers still being present. Interestingly, the same sow would yield different titers through time without a clear pattern making serology interpretation challenging. The period of time that SVA circulates within the herd is still unknown and may play a role as it may lead to re-exposure. qPCR results from oral fluids from the gilts in the growing and finishing pens and processing fluids from the farrowing barns suggest that SVA might have stopped circulating or went undetectable approximately 50 after approximately the outbreak. Interestingly, testing of processing fluids before the onset of clinical disease revealed that the virus had been present in the farm for at least 11 days.

Although the findings of this study are limited to one farm, they do provide information on virus dynamics and antibody persistence, which sheds light on the epidemiologic characteristics of this disease. This study also provides information that will serve for designing a larger scale epidemiological study or an in-farm monitoring program.

Table 1. Number of IgG IFA negative and positive sera samples per week after the outbreak, and number of positive samples per reactive titer.

Week post break	Results		IFA Titer			
	Negative	Positive	1:40	1:80	1:160	1:320
4	9	51	0	3	15	33
8	13	43	5	11	17	10
12	5	45	2	14	23	6
18	13	34	3	7	11	13
20	15	25	0	4	3	18
30	1	42	0	4	5	33

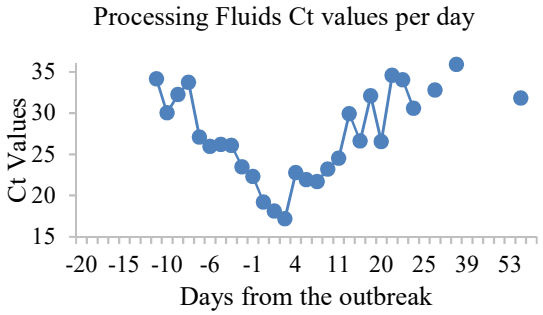

Figure 1. Processing fluids Ct values per day before and after the onset of the SVA outbreak.

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Occurrence of osteochondrosis in pigs the state of Mato Grosso, Brasil.

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Introduction

Osteochondrosis (OC) is a degenerative and non-infectious bone disease, characterized by failure in endochondral ossification (Ytrehus 2007; Olstad et al 2015). OC is known to affect primarily growing pigs with age range of 1 to 8 months, and the disease has been associated with fast growth rate, particular anatomical characteristics, hereditary factors, stress, vascular abnormalities and traumatic lesions (Madson et al 2019). The main clinical sign observed in cases of OC is severe lameness and the most common lesions are noted in long bones of the limbs and in the bones of the pelvis, changes which may be unilateral, bilateral and/or symmetrical (Ytrehus et al 2007; Olstad et al 2015, Madson et al 2019). The objective of the present study is to describe an outbreak of osteochondrosis in pigs in the state of Mato Grosso.

Material and Methods

An on-site visit was carried out in a swine farm located in the state of Mato Grosso, aiming to investigate the occurrence of locomotor abnormalities affecting replacement boars. Affected pigs were submitted for necropsy, tissue samples of several organs were collected, including long bones, and fixed in 10% buffered formalin. Bone samples were decalcified using 10% EDTA. All samples were routinely processed for histopathology, stained by hematoxylin and eosin, and evaluated under light microscopy.

Results

During March of 2019, several replacement boars presented lameness, hind limb paresis, and some affected pigs adopteds back paresis in the visited farm. The prevalence of locomotor deficits was 46%. Affected pigs were part of a group of 50, 7 to 8 months old, terminal boars, which were under quarantine period. These pigs had been purchased from a breeding farm in the state of Minas Gerais, and were transferred to Mato Grosso by road transportation. Clinical signs were observed 12 days after the pigs arrived in the new farm. Including spontaneous death, and euthanasia due to poor prognosis, 15 animals died, which represented a mortality rate of 65%. Out of these 15, four pigs were submitted for necropsy.

Gross lesions were detected in the joints of the pelvic limbs (right and left), and were characterized by abundant yellowish synovial fluid, as well as moderate thickening of the synovial membrane. In the bones, mainly in the head of the femur, multiple depressions of the joint cartilage were noted. In the longitudinal section of these bones, multiple irregular areas were observed in the epiphyseal plates. Furthermore, two pigs also presented complete ischium fracture, which extended to the pubis and sometimes to the ilium. Microscopically, vascular invasion was observed in the joint cartilage, as well as multifocal osteoclastic proliferation in the mineralized cartilage associated with the articular-epiphyseal cartilage complex (A-E complex). In the epiphyseal plate, metaphyseal dysplasia, multifocal areas of cone-shaped thickening in the metaphysis, chondronecrosis (hypertrophic zone) and epiphysiolysis were seen.

Conclusions and Discussion

Clinical and pathologic findings observed in this study are compatible with articular and epiphyseal osteochondrosis, described by Olstad et al (2019) e Madson et al (2019). OC has been described as a multifactorial disorder by Olstad et al (2015). In the present study, it is believed that genetic traits, which favor fast muscular growth and weight gain, associated with biomechanical stress induced by interstate transportation may have played an important role as triggering factors for OC development.

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FOOD SAFETY

Probiotic *Clostridium butyricum* reduces Salmonella serotiters in commercial conditions

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Introduction

Despite multiple large-scale reduction programs, Salmonella remains an important biosecurity risk on-farm. As a result, managing said risk is a continuous effort and requires a holistic approach. Diet origins and ingredients are key factors, to ensure contamination does not enter the farm before production starts. On a more positive note, certain diet ingredients such as zootechnical feed additives can also support the overall biosecurity program: an example hereof are probiotics, viable microorganisms which can confer benefits to the host, if administered in adequate amounts (FAO/WHO, 2002). These benefits go beyond only improving growth performance, depending on which probiotic is used. Recent research has indicated that *Clostridium butyricum* for example is capable to deal with challenges such as Salmonella, which in practice leads to a benefit in terms of securing food safety. To test this in commercial conditions, probiotic *Clostridium butyricum* (strain Miyairi 588, Miya-Gold®) was trialed on-farm, as a tool to mitigate Salmonella infection and proliferation. A commercial fattening farm in Germany was used as trial location, which historically experienced high Salmonella titers at slaughter - despite using an all in – all out batch system. The biosecurity program included in-depth sanitation between batches. Hogs arrived at 30 kg (hybrids coming from the same singular origin farm) and remained on the farm until slaughter. Blood analysis on 60 random samples of an initial group prior to slaughter showed 43 positive results, with an average Salmonella titer of 54.81. As a result, the farm was classified as "Salmonella category III" at the end of 2018, causing meat price deductions in the slaughterhouse.

Material and Methods

The next two consecutive batches of animals received dietary probiotic *Clostridium butyricum* (strain Miyairi 588, Miya-Gold®), for the first batch at a level of 500 g of product/ mton of feed (2.5 x 10^{11} CFU *C. butyricum* Miyairi 588 / mton of feed) for the first week. This dosage was then reduced to 300 g / mton of feed (1.5 x 10^{11} CFU *C. butyricum* Miyairi 588 / mton of feed) for the remainder of the fattening period. The second batch received the same 300 g / mton of feed, but only in the growing stage (30 to 50 kg bodyweight). Before slaughter, 60 blood samples were analysed of each batch. These were recorded as positive if their titer exceeded 40.

Results

Compared to the initial group's 43 positive samples, blood analysis of the first supplemented batch recorded 21 samples as positive. Average Salmonella titer was also lower, measuring 31.13 compared to the initial group's 54.81. This trend continued in the second supplemented batch, with only 16 positive samples. The average Salmonella titer was 26.92, effectively bringing the farm down to a Salmonella Category II.

Discussion and conclusion

Supplementation with probiotic *Clostridium butyricum* Miyairi 588 reduced Salmonella titers, confirming the potential of the probiotic to restrict Salmonella proliferation. As such, dietary probiotic supplementation deserves a place in on-farm food safety programs, reducing the risk of economic deductions at the slaughterhouse due to high Salmonella titers.

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Pig inspection: software for the visual inspection system in pigs

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Introduction

One of the main purposes of meat inspection is to prevent and detect public health hazards, such as foodborne pathogens or chemical contaminants in meat. Furthermore, the information obtained from slaughter animals allows for the planning of animal health management programmes and monitoring the effectiveness of disease treatment and prevention strategies (1, 2). There is a consensus in recognizing that traditional meat inspection is no longer able to address the hazards related to meat consumption. For these reasons, legislations all over the world are changing meat inspection techniques, moving towards visual-only techniques. The visual-only inspection has showed greater efficiency than the traditional inspection in detecting lesions. However, many countries still do not have data on possible applications of a visual inspection system in pigs, moreover consistent data on post-mortem lesions for pigs in the slaughterhouse is missing. There is also great difficulty in the criteria for condemnation of organs or carcass (4, 5). Therefore, the objective of the present study was to develop a software designed to recognize, classify and quantify the main diseases and macroscopic lesions that attack the pigs of age to slaughter in the post-mortem inspection stage.

Materials and Methods

A new protocol of visual-only inspection for pigs was developed based on article 147 of law 29588-MAG-S of Costa Rica, because there were no local visual-only inspection protocols at the time of this study. To give an operative tool to veterinarians, the anatomical structures to be inspected were rearranged into three main groups (head, organs and carcass), which resembles the way organs are found at the end of a slaughtering line.
The scheme was designed to be easily adopted in high production slaughterhouses, shared nationally and comparable with the schemes adopted by the Food Security and Inspection Service in the United States (6). A list of guidelines needed for the univocal interpretation and classification of lesions was developed. The data of each working day is then saved in a file, which can be visible through graphics.

Results

Figure 1 shows the icons for the inclusion of farm data, health inspection data, statistical reports and finally an icon about the software for the user. Figure 2 shows the list of the visual inspection. Figure 3 shows the normal lungs, lungs lesions and the explanation according to the icon selected.

Figure 1. General icons for database.

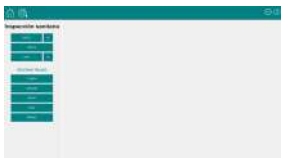

Figure 2. Icons for visual inspection.

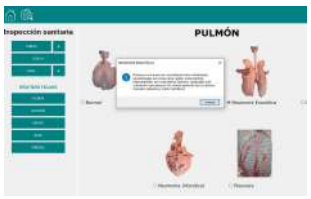

Figure 3. Normal lungs, lungs lesion list and explanation

Conclusions and Discussion

The data derived from local projects on post-mortem lesions in Costa Rican slaughterhouses are not homogenous and comparable. The subjectivity regarding the criteria for condemnation of organs or carcass is an important problem that can lead to significant economic losses to the food industry and producers in general.
For the first time, a classification of lesions was developed in the Costa Rican swine sector based on local legislation and it is also comparable to the schemes adopted by other countries. Moreover, a relevant dataset of these lesions is being built to to assist producers, veterinarians and local authorities with the interpretation of results and the development of health improvement strategies.

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Disseminated mycobacterioses in slaughtered pigs

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Introduction

In a project titled "organ lesions in slaughtered animals" financed by the Federal Food Safety and Veterinary Office meat inspectors can submit organ lesions for pathological, bacteriological and parasitological examination to train their "diagnostic competence".
Between early 2017 and the end of October 2019, 213 pig organ submissions were received. Of these a mycobacteria infection (MI) could be demonstrated or was suspected in 14 cases (6%).

Materials and Methods

The organs were examined macroscopically and histologically. When granulomatous lesions were present, Ziehl-Neelson staining was carried out. In 7 of the 14 cases with suspected MI fresh material was used for bacteriological examination by culturing in liquid and solid mycobacterial media. Species identification was performed using PCR and sequencing in 2 cases or matrix-assisted laser desorption/ionization time-of flight mass spectrometry (MALDI-TOF-MS) in 5 cases (1).

Results

Out of these 14 cases, only 3 showed exclusively granulomatous or caseous lymphadenitis of the digestive tract, the most common manifestation of MI in pigs (2). In the remaining 11 cases (79% of the MI cases) other organs were also sent in, representing more disseminated or generalized pattern of mycobacterioses (3). Liver und lung were affected in 8 cases, spleen in 4, kidney in 2 and pleura lesions were observed in 2 cases. In 10 cases, more than one visceral organs were affected. Out of seven molecular investigations, 2 PCRs combined with sequencings revealed *Mycobacterium avium* spp. *hominissuis* (MAH) and the 5 MALDI-TOF analyzed cases revealed the species *Mycobacterium avium,* most likely the subspecies *hominissuis*.
Gross lesions of lymph nodes ranged from enlargement alone to small spotted necrotic or calcified areas to larger areas with calcification or extended caseation. In livers lesions could be very small and spotted or more extensive with a 'milk-spot' like appearance. In spleen dark red small nodes could be present, whereas in lungs few to many whitish nodules or confluent whitish regions were the suspicious lesions. Two cases with kidney lesions showed extensive beige white foci similar to large multifocal interstitial nephritis or leucosis respectively. Examples of macroscopic lesions are the topic of the poster.
In histology the mycobacterial changes covered a wide range of granulomatous stages from exudative to fibrotic reactions and mixed lesions (3). However, a simple assignment of the lesions to the four stages of focal granulomatous reactions as defined in bovine tuberculosis (4) was not possible because in our cases the inflammatory processes were more of multifocal coalescing character. In all but one case (only lymph node with encapsulated tubercles with central necrosis and calcification, no giant cells) abundant multinucleated giant cells of the foreign-body- or Langhans-type were present, which was often the diagnostic clue for the assessment of a mycobacteriosis, especially in the visceral organs. An accumulation of eosinophils was present in many cases. Acid-fast bacteria could be detected in very small amounts in 13 cases. Bacteria were found in the visceral organs (in 9 cases) as well as in lymph nodes, mainly in giant cells from the Langhans-type.

Conclusions and Discussion

In all seven cases subjected to molecular investigation, *Mycobacterium avium* was detected most likely spp. *hominissuis* (MAH). The name "hominissuis" designates the two main species of this opportunistic pathogen, namely humans and pigs. MAH occurs ubiquitously in the environment and environmental contaminations seems to be the main infection source for both species, pigs (for example bedding material) and humans (water systems, sauna pools etc.) (4).
In Switzerland the traditional meat inspection is still performed and in disseminated MAH infections (visceral organ involvement additional to lymph node lesions) the whole carcass has to be condemned. If lymph nodes alone are affected, the carcass is passed as fit for human consumption.
In conclusion, the presence of enlarged or necrotic or calcified lymph nodes of the digestive tract together with whitish "milk spot" like lesions in liver, eventually combined with whitish lesions in lungs or reddish nodules in spleen, should be interpreted as generalized mycobacteriosis (5) with subsequent condemnation of the carcass. Alternatively, the presence of strange 'milk-spot' lesions in the liver should lead to a close inspection of the lymph nodes of the digestive tract.

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Efficacy of overgrown rye fermented with *Agaricus subrufescens*prototype productsand organic acids to reduce *Salmonella*Typhimurium: a meta-analysis

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Introduction

Salmonellosis in swine is a zoonoses and a global concern. Contamination risk exists at different levels of theproduction chain(1). Global antibiotic reduction requires new relevant strategies to reducezoonoses in the feed-to-food chain.Organic acids (OA) are used in nursery pig dietsand appear promising for *Salmonella* control (2). Edible mushrooms (i.e. sun mushroom) and derivate products possessing antimicrobial and immunomodulatory properties (3,4)may be a potential solution (5). Fungal bioactive components include 1,3-1,6-β-glucans, glycoproteins, vitamins, prebiotic oligosaccharides and others (4).

Materials and Methods

The present objective was to assess dietary treatments: 1) Control, 2) 4 kg/t OA blend (OA, formic and lactic acid), 3) OA + 2 kg/t fungal prototype A (FryeA, fermented rye with *Agarics subrufescens*), and 4) OA + 2 kg/t fungal prototype B (FryeB, fermented rye with *Agarics subrufescens* based product) on weaned pigs challenged with *Salmonella*Typhimurium (S. Typh).

A meta-analysis was performedincluding three Salmonella challenge studies (A, B, C; see Table 1) conducted in Trouw Nutrition R&D facilities (The Netherlands). Studies used individually penned pigs challenged orally for 7 consecutive days with ~9.0 $\log_{10}$ CFU S.Typh/dayaccording to Litjens et al. (6).

Table 1. Description for studies used.

Study	Pigs / treatment	BW at weaning	Challenge day post-weaning	Study length
A	8	7.12 kg	day 13	20 days
B	12	7.37 kg	day 8	21 days
C	12	7.20 kg	day 5	17 days

Individual fecal samples were collected pre- and post-challenge on days 1, 2, 3, 4, and 7 equally among studies. Overall average daily gain (ADG), feed intake (ADFI), feed efficiency (FE), area under the curve (AUC) for S. Typh shedding in feces(up to day 4 and 7 post-challenge), peak of S. Typh shedding, sum of diarrhea scores (1-3),and severe diarrhea (score 3) as frequencywithin 7-day challenge were used in the meta-analysis. The MIXED Model procedure (PROC MIXED, SAS Inst. Inc., Cary, NC)was used including treatment as main effect, within study variation as a random effect, and study (A, B, C) as a repeated effect.

Results

After challenge, S. Typh shedding was detected and included a peak within 2, 3, and 4 days post first inoculation.Among studies, B and C were consistent on treatment effect while study A was not shown with differences. Shedding peak of S. Typh and shedding AUC at 4 and 7 days post-challenge were lower ($P< 0.05$) in pigs fed FryeA and FryeB compared withcontrol. Furthermore,FryeA showed a tendency ($P< 0.10$) for lower values than OA supplemented alone (Table 1). There were no differences observed for performance and diarrhea frequency among treatments.

Table 2. Meta-analysis main variable responses for pigs 7-day challenged with ~9.0 $\log_{10}$ CFU S. Typh

	Shedding peak, $\log_{10}$ CFU/g feces	Shedding AUC 7days, $\log_{10}$ CFU	Severe diarrhea 7-day,%	ADG total period	ADFI total period
Control	5.89^{b}	29.6^{b}	15.8	265	314
OA[1]	5.32^{aby}	27.2^{aby}	12.3	252	296
FryeA[2]	5.19^{ax}	25.7^{ax}	12.7	256	314
FryeB[2]	4.89^{a}	23.5^{a}	10.9	267	322
SEM	0.277	1.014	4.03	20.6	19.7
P-value	0.005	0.001	0.676	0.968	0.563

[1]OA= 4 kg/t organic acid blend (formic and lactic acids);
[2]FryeA and FryeB dietary treatments include OA + 2 kg/t fungal prototype of fermented rye with *Agarics subrufescens* fungi based product.
[x-y]Different superscripts tend to differP<0.10.
[a-b]Different superscripts differ P< 0.05.

Conclusions and Discussion

Mechanisms of action in reducing shedding may include S. Typh affinity to bind fermented rye oligosaccharides (5) and immune modulation (4).

Fermentedrye including*Agaricus subrufescens*combined with organic acids showed potential to reduce *Salmonella*Typh infection and shedding, and FryeA tended to improve these metrics compared to organic acidalone. These results justify additional research with fungal fermented products on *Salmonella* control.

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Case Report: Reducing antibiotic use while controlling *Salmonella* prevalence in Weaning Piglets in a commercial farm

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Introduction

Research for new strategies that promote gut health in pigs is key because there is an urgent need to reduce antibiotic usage in production animals and minimize risk of bacterial resistance against antibiotics. Salmonellosis, although often asymptomatically in pigs (1), is a major human concern globally because contamination can occur at many different levels of the pig production chain [Regulation (EC) No. 2160/2003]. Reducing *Salmonella* prevalence in pork production is also of importance for pig or pork exporting countries. It has frequently been demonstrated that the use of antimicrobial agents in production animals favors the development of resistance among foodborne pathogens like *Salmonella* spp. (2). Therefore, there is an urgent need for new antibacterial strategies to reduce Salmonellosis. Due to their bacteriostatic and bacteriolytic properties, organic acids are frequently used to control *Salmonella* by increasing stomach barrier function by reducing stomach pH and destabilize bacterial membrane function resulting in bacterial cell death. This case report analyses the ability of an integrated approach, including nutritional, farm management and health strategies to control *Salmonella* infection in a commercial farm, improving animal performance and reducing the use of antibiotics.

Materials and Methods

The study was conducted at a commercial farm with 500 sows in Colombia from 2018 until November 2019. The farm presented severe cases of diarrhea, mortality and low productive performance in weaned piglets. A molecular typification of *Salmonella* spp. with a phylogenetic analysis MEGA7 was performed. A total of 26.6% colonies were positive to enteric *Salmonella* serovars Heidelberg, *Paratyphi*, and Typhimurium. Different control strategies were implemented with antimicrobial interventions via feed and parenteral during 2018. The treatments were as follows: 1) in 2018 use of antibiotic growth promotor (AGP) Colistine Sulphate at 40 ppm; 2) in 2019 the AGP was replaced by a synergetic Organic Acid (OA) blend at 3 kg/t + TriBasicCopper-Chloride 150 ppm (TBCC). Animal performance parameters were compared over the 2 years; where no changes were made in genetic selection.

Results

Performance results show a numerical improvement in performance (Table 1). From 2018 to 2019, there was an increased number of weaned piglets per sow per year which let to have a difference in kg of weaned piglets per sow per year (2018: 868.52 kg piglet/sow/year; 2019: 957.67 kg piglet/sow/year). However due to a reduction in percentage of mortality in 2018 each sow produced an average 23.97 kg of piglet less, while in 2019 was 9.57 kg of piglet less.

Table 1. Technical parameters (annual average):

Parameters	**2018**	**2019***
Farrowing rate (%)	90.0	92.2
Weaned piglets / sow / year	28.3	31.72
Average Weight at Weaning at 21 days (kg)	5.57	5.18
Average Weight at End of Weaning (kg)	30.69	30.19
ADG weaners (kg/d)	0.485	0.495
FCR	1.47	1.38
Mortality at End of Weaning (%)	2.76	1.00

*2019: data from January to November

Conclusion and Discussion

The case study shows the possibility to have a positive impact in technical performance parameters, while reducing the use of antimicrobials in swine production during a period of controlling *Salmonella* in traditional farms with sanitary challenges.

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An analysis of *ymoA* gene expression in *Yersinia enterocolitica* strains with different enterotoxic properties, isolated from pigs

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Introduction

Yersiniosis caused by *Yersinia enterocolitica* is one of the most reported zoonoses in the EU (1) with pigs being the most important source of infection (2). Strains isolated from humans with clinical yersiniosis and diarrhoea are able to produce enterotoxins YstA (*Yersinia* stable toxin A) (3). YstA is encoded by the *ystA* gene, but not all *ystA*-positive strains produce enterotoxins. Some authors suggested that the absence of enterotoxic properties in selected *Y. enterocolitica* strains could result from the inhibitory influence of the *ymoA* gene (4,5). Aim of this study was an analysis of *ymoA* gene expression in *Y. enterocolitica* strains with different enterotoxic properties, isolated from pigs.

Materials and Methods

Y. enterocolitica strains were isolated from fattening pigs without clinical signs of yersiniosis, belonged to bioserotype 4/O:3 and were *ystA*- and *ymoA*-positive. *Y. enterocolitica* strains producing enterotoxin YstA in the suckling mouse bioassay formed Group I, and *Y. enterocolitica* strains not producing enterotoxins in the suckling mouse bioassay formed Group II.

The expression of *ystA* and *ymoA* was normalized in relation to the *gapA* and *polA* reference genes using quantitative real-time PCR (qPCR). Sequences of the primers used in the study are shown in Table 1. The reaction mixture contained cDNA, primers and the QuantiTect SYBR Green RT-PCR master mix (Qiagen, Hilden, Germany). Amplification curves were generated from real-time qPCR data. The CT was calculated based on a fluorescence threshold and the ΔCT for each sample was created using the equation *ΔCT = CT target gene—CT reference gene* to calculate the expression of each gene.

Table 1. Sequences of the primers used in the study

GENE	PRIMERS SEQUENCES
ystA	5'GTCTTCATTTGGAGGATTCGGC3' 5'AATCACTACTGACTTCGGCTGG3'
ymoA	5'GACTTTTCTCAGGGGAATAC3' 5'GCTCAACGTTGTGTGTCT3'
polA	5'GCTGGCTTGCGGATGTAGAT3' 5'AGCACGGCGGTCACTTCA3'
gapA	5'CCATCCGTGTTACCGCAGAG3' 5'TCTTAGCACCAGCAGCAATGT3'

Results

Statistically significant differences were not observed in both, Group I and Group II of strains isolated from pigs. In the group of *Y. enterocolitica* strains isolated from pigs and not capable of producing enterotoxin YstA, the relative expression level of the *ymoA* gene was higher than the expression level of the *ystA* gene, but the observed differences were not statistically significant. Significant differences in the relative expression levels of *ystA* and *ymoA* genes were not noted in *Y. enterocolitica* strains isolated from pigs and capable of producing enterotoxin YstA in the suckling mouse bioassay. Therefore, no correlation was found between the relative levels of *ystA* and *ymoA* mRNA in *Y. enterocolitica* strains isolated from pigs and producing enterotoxin YstA in the suckling mouse bioassay, as well as in *Y. enterocolitica* strains not producing enterotoxin in this test.

Conclusions and Discussion

An analysis of *ymoA* gene expression in *Y. enterocolitica* strains isolated from pigs showed that this expression did not affect *ystA* gene expression and the ability of the strain to enterotoxin YstA production.

Acknowledgments

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Thymol, Carvacrol and Sorbic Acid as Effective Tools Against *Salmonella typhimurium*

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Introduction

Salmonella typhimurium (ST) is a foodborne pathogen, with pigs being one of the major routes of transmission to humans (1). Since botanicals and organic acids can be used for the prevention of intestinal colonization by foodborne pathogens (2), the aim of this study was to test the ability of thymol (THY), carvacrol (CAR), and sorbic acid (SOR) in controlling a ST intestinal challenge *in vitro*.

Materials and Methods

First, a minimal inhibitory concentration (MIC) test and a time-kill assay were performed. *S. typhimurium* ATCC® 6994™ was cultured at 37°C in BHI. For the MIC assay ST (10^6 CFU/mL) was tested over a range of serial dilutions of THY, CAR, and SOR. In the time-kill assay, the same concentration of bacteria was grown in presence of THY 0.94 and 1.87 mM, CAR 0.94 and 1.87 mM, or SOR 50 and 100 mM. Bacterial growth was estimated by plate counting at inoculation (0 h), and then every hour for 8 time points and 24 hours post-inoculation.

Second, intestinal Caco-2 cells were seeded on 3 µm transwell inserts and allowed to grow in DMEM + 10% FBS at 37°C (5% CO_2), until stable. Then cells were challenged with ST (10^5 CFU/mL) without any treatment (challenged control, CTR+) or with either THY 0.47 mM, CAR 0.47 mM, or SOR 25 mM, (n=5). A group without bacterial challenge was the non-challenged control (CTR). Trans-epithelial electrical resistance (TER) was measured at 0 h, 2 h, and 4 h post-challenge. Data were analyzed with 2-way ANOVA and differences considered significant at $P<0.05$.

Results

MIC values for THY, CAR, and SOR were 1.87 mM, 1.87 mM and 100 mM, respectively. Time-kill results are shown in Figure 1. Both THY and CAR 1.87 mM killed ST immediately after inoculation, while the half doses showed a reduction in number of viable cells over the first 8 h, reaching the time-kill at 24 h post-inoculation. Counts of bacteria cultured with SOR at both concentrations tested did not vary over time.

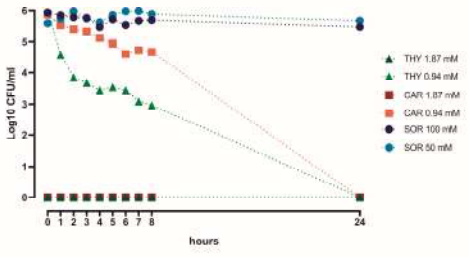

Figure 1 Time-kill assay performed with THY, CAR, or SOR against *S. typhimurium*.

About Caco-2 cells, TER results are shown in Figure 2. After 2 h, THY and CAR 0.47 mM were already able to increase TER compared to both CTR+ and CTR- ($P<0.0001$). Then, 4 h post-challenge, CTR+ group showed a drop in TER, whereas all treated cells had significantly higher TER compared to CTR+ ($P<0.05$). Moreover, THY and CAR maintained a higher TER value also compared to the non-challenged CTR ($P<0.0001$).

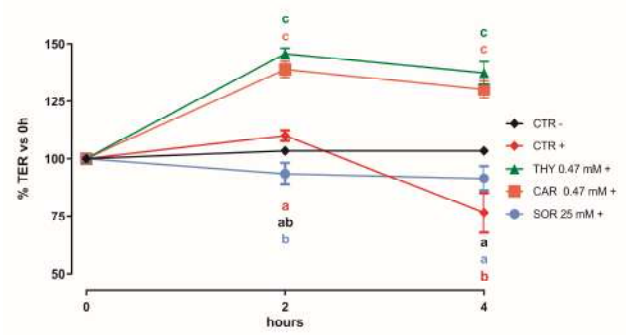

Figure 2. TER of Caco-2 cells cultured with THY, CAR, or SOR at 2 and 4 h post-challenge with *S. typhimurium*. Data are presented as mean ± SEM. Within a time point, each treated group was compared to both control groups. Different letters indicate statistical significance with $P<0.05$.

Discussion and Conclusions

Based on time-kill results, thymol and carvacrol showed a bactericidal action against ST in a dose-dependent manner, while sorbic acid was bacteriostatic, confirming the MIC data and the antimicrobial action of these molecules.

Moreover, the treatments were effective in preserving intestinal Caco-2 cells under a ST challenge. Sorbic acid was able to maintain the monolayer integrity despite the bacterial challenge, while thymol and carvacrol quickly and strongly increased Caco-2 integrity, also compared to the non-challenged cells.

In conclusion, thymol, carvacrol and sorbic acid showed a direct antimicrobial effect against *S. typhimurium* and were also effective in protecting intestinal cells under the bacterial challenge. Their inclusion as active ingredients in feed additives can be proposed as a valid tool to control *Salmonella* in pigs.

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Prevalence and antimicrobial resistance of *Salmonella* from raw pork sold at butcher shops and supermarkets in Chiayi City, Taiwan

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Introduction

Pork is considered to be a source of human salmonellosis in many countries. *Salmonella* has also been shown to contaminate pork during slaughter through affected pigs, contaminated environments or equipment and raw pork sold by retailers may be a source of consumers to exposure to *Salmonella* (1). On the other hand, widespread of antimicrobial resistant *Salmonella* has been a serious global human and animal health problem. The purpose of this study is to investigate the prevalence of *Salmonella*, serotype and antimicrobial resistance of the *Salmonella* isolates in raw pork from butcher shops and supermarkets in Chiayi City to understand food safety situation in Taiwan.

Materials and Methods

Raw pork samples (n=60) were collected from 5 butcher shops (B1-B5) and 5 supermarkets (S1-S5) in Chiayi City during December 2019. Each market was collected 6 different raw pork samples. Isolation of *Salmonella* was carried out based on Bacteriological Analytical Manual formulated by USDA/FSIS (2). 25 g of pork was added to 225 ml BPW, Rappaport-Vassiliadis R10 broth (R10 broth), modified semi-solid Rappaport-Vassiliadis agar (MSRV) and brilliant-green phenol-red lactose sucrose agar (BPLS) were chosen as the selective culture medium and differential medium. *Salmonella* spp. was finally confirmed by polymerase chain reaction (PCR).

Serotype was identified by pulsed field gel electrophoresis (PFGE). *Salmonella* serotype Braenderup H9812 strain was used as the molecular size standard.

Antimicrobial resistance of *Salmonella* was determined by disk diffusion method, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923 were used as quality control standard strains (1). Selection of antibiotics is based on clinically using in veterinary and human medicine, including ampicillin (AMP, 10μg), ceftiofur (FUR, 30μg), chloramphenicol (C, 30μg), gentamicin (CN, 10μg), tetracycline (TE, 30μg), doxycycline (DO, 30μg), nalidixic acid (NA, 30μg), trimethoprim-sulphamethoxazole (SXT, 1.25/23.75 μg), ciprofloxacin (CIP, 5μg) and colistin (CS, 10μg).

Result

3 raw pork samples from supermarkets (3/30) and 5 from butcher shops (5/30) are positive of *Salmonella*, and the isolation rate in this study is 13.3% (8/60). *Salmonella* isolates in this study exhibit resistance to chloramphenicol (5/8), colistin (1/8), ampicillin (5/8), trimethoprim-sulphamethoxazole (5/8), tetracycline (5/8) and doxycycline (2/8) (1). 4 serotypes of *Salmonella* was identified in raw pork, including *S.* Kedougou (2/8), *S.* Typhimurium (2/8), *S.* Derby (1/8) and *S.* Anatum (1/8), and the others are unidentified (2/8).

Table 1. Serotype and antimicrobial resistance of *Salmonella* isolates in this study

No.	Origin	Serotype	Antimicrobial resistance
1	S3	Kedougou	-[1]
2	S3	Kedougou	-
3	S4	Derby	-
4	B3	Typhimurium	C, AMP, SXT, TE
5	B3	Anatum	CS, C, AMP, SXT, TE, DO
6	B4	Typhimurium	C, AMP, SXT, TE, DO
7	B4	Unidentified	C, AMP, SXT, TE
8	B4	Unidentified	C, AMP, SXT, TE

[1]Not present resistance to the antimicrobial agents tested.

Conclusions and Discussions

Salmonella was detected in raw pork both in supermarkets and butcher shops, which means that consumers need to pay attention to handling or eating pork from supermarkets as from butcher shops, although pork from supermarkets usually has more safety check. 5 of 8 *Salmonella* isolates showed resistance to four or more antimicrobial agents tested, and these *Salmonella* isolates are resistance to more than three antimicrobial categories, which conforms to the definition of multidrug-resistant (MDR). Therefore, more hygiene management and education of food safety are needed for retailers and consumers to reduce the risk of infection.

Further study includes expanding the sampling area to increase the sample number and carrying out antimicrobial resistant gene identification.

Acknowledgments

Animal Disease Diagnostic Center of National Chiayi University.

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Effect of the fruit aqueous extract of Brazilian pepper tree (*Schinus terebinthifolius*, Raddi) on lipid peroxidation of frozen fresh pork sausage

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Introduction

Consumption of fresh sausages has increased in recent years, mainly because they are simple, quick to prepare. However, the consumption of meat products has been associated with obesity, cancer, and heart disease, raising public health concerns about safety, sanitation, convenience, and flavor of foods (1). The main problem in the processing and storage of fresh sausages is the lipid peroxidation (2). Consequently, to improve the quality of meat products and gain the consumer's preference, the main innovation in the meat producing chain today is the replacement of synthetic preservatives and antioxidant agents by natural products that have the same effect on foods (3). Therefore, the objective of the present study was to determine lipid peroxidation of frozen fresh pork sausage prepared with aqueous extract of *S. terebinthifolius* seeds (AESt) as a natural ingredient with antioxidant properties in foods.

Materials and Methods

AESt was prepared according to (4). Briefly, S. terebinthifolius fruits were picked and washed. Next, 100 g of fruits were weighed and blended with water (1:1) in a food processor. The juice formed was filtered, frozen, and lyophilized in a benchtop freeze-dryer (L202, Liotop).

After adding mixture of curing salts and AESt, fresh sausage material was placed in other trays, labeled, and refrigerated for 1 h at 2ºC to 4ºC. Sausages were manually encased using 36-mm natural sheep casing. The sausages prepared were vacuum-packaged (TM720, TecMaq) in polyethylene bags, labelled, stored at -18ºC, and thawed at 4ºC before analyses.

Three sausage formulations were prepared containing different amounts of AESt (0.25%, 0.50%, and 1.0%, called T2, T3, and T4, in that order). A control formulation (T1) was prepared using sodium erythorbate 0.025%, which is the usual additive in meat products .

The experimental design used was completely randomized with repeat time measures, three repeats, and four formulations. The data obtained were submitted to an analysis of variance (ANOVA) in the software Proc Mixed (SAS® Enterprise Guide 4.3, Cary, NC, USA). Means were estimated using the LSMeans test at a 5% probability level.

Results

Mean values of mg MDA/kg in fresh pork sausages during freeze-storage are shown in Table 1. In the beginning of the experimental period, TBARS levels did not vary significantly ($p > 0.05$) between T1, T2, and T3 (0.084 ± 0.040, 0.104 ± 0.049, and 0.047 ± 0.022, respectively). However, with mean TBARS level of 0.029 ± 0.014, T4 differed significantly from T1 and T2 ($p < 0.05$). On day 45 into the experiment, TBARS values of T3 also began to differ significantly from T4 ($p < 0.05$). Mean TBARS values of formulations T1, T2, and T3 did not differ throughout the experiment ($p > 0.05$), showing that the addition of AESt 0.25% and 0.50% had a similar antioxidant effect compared with T1, which contained sodium erythorbate alone. In turn, AESt 1.00% (formulation T4) was more efficient at inducing antioxidant effect than the addition of sodium erythorbate alone (T1), 0.25% AESt (T2), and 0.50% AESt (T3).

Conclusions and Discussion

T4 was the formulation that best resisted lipid peroxidation, with a better conservation profile and increased shelf life.

Table 1. TBARS levels (mg MDA/kg) of fresh, freeze-stored pork sausage formulations prepared with different levels of aqueous extract of *S. terebinthifolius* during storage.

TIME (DAYS)	TREATMENT[1] T1	T2	T3	T4
0	0.084^{Aa}	0.104^{Aa}	0.047^{ABa}	0.029^{Ba}
45	0.587^{Ab}	0.620^{Ab}	0.611^{Ab}	0.445^{Bb}
60	1.308^{Acd}	1.046^{Ab}	0.974^{Ab}	0.516^{Bb}
90	1.766^{Ad}	1.845^{Ac}	1.854^{Ac}	1.799^{Ac}
120	2.080^{Ad}	2.115^{Ac}	2.159^{Ac}	1.489^{Bc}
420	2.006^{Ad}	2.071^{Ac}	1.848^{Ac}	1.413^{Bc}

[1]Means followed by identical uppercase letters on the same line and identical lowercase letters on the same column do not differ statistically ($p > 0.05$).

The addition of AESt 1.0% delayed lipid peroxidation and interrupting oxidation reactions early into storage, thus increasing shelf life. The antioxidant effect induced by the inclusion of AESt 0.25% and 0.50% was similar to that exhibited by sodium erythorbate.

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Effect of the extract of balloon pepper (*Capsicum baccatum* var. pendulum) on lipid peroxidation of fresh pork sausage and smoked

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Introduction

The reduction of the quality of foods usually associates with the deterioration of their compounds. Lipid oxidation is the main reason for this deterioration (1). Oxidation processes affect the nutritional, sensorial values of the products, and the level of thiobarbituric acid reacting substances (TBARS), which is the main parameter to assess the lipid oxidation (2). In this context, studies involving the effect of time and storage conditions of meat products are essential factors in meat processing, since problems related to storage stability are common (3). Therefore, this study aimed to assess the influence of the use of natural antioxidants based on extracts of the balloon pepper (*Capsicum baccatum* var. pendulum) on the oxidation of fresh pork sausage and smoked.

Materials and Methods

The extraction method was adapted from (4), choosing for the production of oleoresins. In the beginning, the fruits were cut to extract the seeds and washed with tap water, weighed, and placed on an air-circulation oven at 40ºC, until reaching a constant mass. The ethanol was the solvent used for the preparation of the extraction. After the drying, fruits were minced and submitted to extraction in the Soxhlet equipment, using ethanol 70% (Fruit-solvent ratio: 1:10 weight/volume) for four hours. The extracts were evaporated by a rotary evaporator at 79ºC. After this process, the antioxidant extract of the balloon pepper was obtained.

Five different formulations of fresh pork sausage and smoked sausage were produced, for the exclusive study of lipid oxidation: Positive control, F1 (negative control), F2 (0.5%), F3 (1%), and F4 (1.5% of antioxidant extract of the balloon pepper). The formulations were produced promoting the substitution of the synthetic antioxidant with the natural one. A positive control using sodium erythorbate, and a negative control without any antioxidant product were included in the experiment. After their production, the sausages were vacuum-sealed and stored at -18ºC for further analyses of lipid oxidation.

The results were submitted to Analysis of Variance (ANOVA) at 5% probability. The treatments were compared by the test Student-Newman-Keuls (SAS, 2009 version 9.3).

Results

Table 1 present the values of TBARS obtained. We did not find significant differences (P>0.05) among the treatments, and at different times (storage). TBARS values ranged between 0.013 and 0.065 mg MDA/kg of the sample, respectively at 15, and 60 days of storage. These data display the similarity between the action of the natural antioxidant and the sodium erythorbate. Smoked sausage samples also did not display significant differences (P>0.05) associated neither to the treatment nor the storage. TBARS values ranged between 0.013 and 0.069 mg MDA/kg of sample along the experimental period. This result may be associated with the degradation of MDA to other compounds due to the action of phenolic compounds produced during the smoking process.

Conclusions and Discussion

We do not see the effects of formulas upon rancidification, once storage by freezing and vacuum packaging contributed positively to the oxidation despite the use and type of antioxidant.

Table 1. Average values of TBARS expressed as MDA/Kg found by lipid oxidation in fresh and smoked pork sausage formulations with the use of the balloon pepper.

FRESH	TBARS (DAYS)[1]			
	0	15	30	60
Control	0.004[a]	0.015[a]	0.026[a]	0.038[a]
F1	0.020[a]	0.013[a]	0.025[a]	0.059[a]
F2	0.030[a]	0.020[a]	0.024[a]	0.057[a]
F3	0.026[a]	0.024[a]	0.028[a]	0.065[a]
F4	0.036[a]	0.017[a]	0.024[a]	0.062[a]
SMOKED	TBARS (DAYS)[1]			
	0	15	30	60
Control	0.058[a]	0.026[a]	0.060[a]	0.046[a]
F1	0.030[a]	0.014[a]	0.038[a]	0.049[a]
F2	0.066[a]	0.016[a]	0.035[a]	0.038[a]
F3	0.064[a]	0.013[a]	0.069[a]	0.042[a]
F4	0.042[a]	0.019[a]	0.047[a]	0.064[a]

[1]Averages on the same line and column, followed by lowercase letters, not differ by SNK test (p<0.05).

Although fresh sausage samples showed higher values of lipid oxidation compared to smoked samples, the response during 60 days of storage was similar.

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GENETICS

Population Genetic Structure in Feral Pigs (*Sus scrofa*, Artiodactyla: Suidae) from in the Brazilian Pantanal biome

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Introduction

The introduction of exotic ungulates worldwide has been particularly destructive due to their strong ability for occupying terrestrial ecosystems, resulting in extensive damage to native faunas and floras. In Pantanal, the feral pigs "porco monteiro" live in sympatry with the native, white-lipped peccary, *Tayassu pecari* and the collared peccary, *Pecari tajacu* (1). In this study, we used 12 microsatellite markers for assessing the genetic diversity and population structure of feral pigs from two regions of the South American Pantanal biome.

Materials & Methods

Specimens were collected in the southern Pantanal biome (Mato Grosso do Sul state, Brazil), in subregions of Nhecolândia and Rio Negro, belonging to two localities in the municipalities of Corumbá and Aquidauauna, approximately 135 km apart. In the subregion Nhecolândia, 23 specimens were collected and in the subregion of Rio Negro, 81. DNA was isolated from tissue preserved in 100% ethanol following standard procedures and Twelve polymorphic microsatellites were PCR amplified using *TNFB, SW72, IGF1, SW445, SW857, SW24,*
SW240, SW444, SW2008, ACTG2, S0007, and PGHAS primers. Genetic diversity was estimated using GENEPOP 4.2 and Allelic richness (AR) was estimated with FSTAT 2.9.3.2 Global and population-specific tests for deviations from Hard-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) among loci were performed with ARLEQUIM 3.5.1.2. Inference on the degree of genetic differentiation among predefined geographic groups was investigated with pairwise Fst measures implemented in ARLEQUIN and with the related Rst index. The Bayesian clustering approach implemented with STRUCTURE 2.2. Evidence of recent population bottlenecks was investigated by testing excess of heterozygosity using Bottleneck. LDNE was used for estimating Ne from genotypic data based on the LD method.

Results

Significant deviations from HWE induced by lack of heterozygotes were observed at two loci: *SW24* and *SW2008* after the Bonferroni correction ($\alpha = 0.05$) and were therefore excluded from analyses. The two populations were polymorphic at all loci, with 2-11 alleles per locus in Nhecolândia and 3-17 in Rio Negro. The average number of private alleles accounted for 1.0 in Nhecolândia and 1.4 in Rio Negro. The moderate levels of genetic diversity were not significant for allelic richness ($Z = 1.61$, $P = 0.11$) or for observed ($Z = 0.75$, $P=0.45$) and expected ($Z = 0.16$, $P = 0.87$) heterozygosy.
The Fst estimate (0.0238) was significantly different from 0 ($p< 0.0001$) as well as Rst (0.0244; $p< 0.0001$). Genetic clustering analysis, carried out with STRUCTURE, showed the lowest likelihood and variance values with K = 1 [Mean L (K) = -2498.5/ Mean Var = 33.0], supporting a most probably single cluster.
Estimates of excess of heterozygosity showed no evidence of either recent ($P = 0.75$) or long-term bottlenecks. The effective population size (Ne) was estimated as 103 individuals (ranging from 60 to 240).

Discussion and Conclusions

The existence of genetic flow between these populations together with the frequent genic influx from crossings with domestic pigs ensures the perpetuity of these feral and invasive swines in the Pantanal. The maintenance of feral pigs populations in the Brazilian Pantanal pose far-reaching sanitary an ecologic challenges beacause they are reservoirs of infectious agents capable of infecting human, wildlife and domestic species (2,3) and their capacity of efficiently competing for natural resources with wildlife (1).

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Geographic and Phylogenetic Distribution of PRRSV in México

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Introduction

The Porcine Reproductive and Respiratory Virus (PRRSV) genome is a single-strand positive sense RNA of 15 kb in length, containing several structural and non-structural proteins. Among those proteins, GP5 is a structural glycosylated protein and the most variable of PRRSV[1]. ORF5 sequences from PRRSV type 2 have been grouped per lineages and sublineages[2] that can be used as a reference to determine diversity of PRRSV isolates by regions or countries. The objective of this study was to sequence and analyze the ORF5 segment in PRRSV isolates obtained in different regions of Mexico during 2015–2019, thus documenting the genetic variation in the circulating variants of PRRSV according to their different linages and assisting in the understanding of the patterns of geographical distribution of this virus in Mexico.

Materials and Methods

Three hundred and ninety seven samples (sera, oral fluid, processing fluids and tissue swabs) from 13 Mexican states (Aguascalientes, Estado de México, Guanajuato, Hidalgo, Jalisco, Michoacan, Nayarit, Nuevo Leon, Puebla, Queretaro, Sonora, Tamaulipas y Veracruz) were analyzed to identify ORF5 PRRSV during June 2015-March 2019. RNA extraction was made with QiAmp Viral RNA mini Kit catalogue number 52906. ORF5 sequencing was carried out using the Miseq System equipment, while the nucleotid sequence analysis was performed with MEGA 7.0 software[3]. The phylogenetic tree was built applying the Neighbor Joining building model and a 1000 repetitions Bootstrap analysis. A map showing the geographical distribution of the different PRRSV lineages by state in Mexico was generated using ArcMap 10.7.1 (ESRI, Redlands, CA).

Results

Published references at GenBank from the nine PRRSV type 2 ORF5 lineages were compared to the 397 sequences on file and a phylogenetic tree (figure 1) was generated applying MEGA 7.0 software. The tree in figure 1 shows that 161 sequences or 41% of the total belong to the lineage 1 (L1), 40 sequences (10%) to lineage 2 (L2), 125 sequences (31%) to lineage 5 (L5), 2 sequences (1%) to lineage 7 (L7) and 69 sequences (17%) to lineage 8 (L8).

The map in figure 2 shows the distribution of L1, L2, L5, L7 and L8 by state in Mexico. Six of the 13 states in the database represent 70% of the volume of pork production in Mexico[4].

Conclusions and Discussion

This study analyzed the genetic variability of different PRRSV isolates in several states from Mexico, based on the system of lineages published by Shi *et al*[2]. The resultant phylogenetic tree shows that the main lineages on this Mexican database are L1 and L5 (MLV vaccine-related), consisting of 72% of the 397 PRRSV isolates in study, while L2 and L8 account for 27% of the isolates and only 2 sequences (1%) belong to L7. The findings from this study show a nationwide distribution of lineages L1, L5 and L8, stressing the need for a better understanding of pig movements between regions in Mexico.

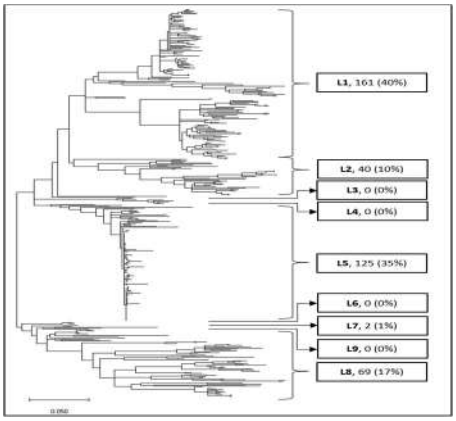

Figure 1. Phylogenetic tree of 397 PRRSV Type 2 ORF5 sequences from Mexico containing isolates from 5 differents lineages (L1, L2, L5, L7 and L8).

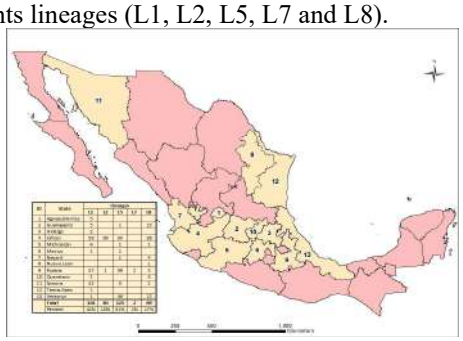

Figure 2. Map showing the geographical distribution by state of the PRSV type 2 ORF5 lineages detected in Mexico.

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Correlation estimates for alternative litter performance traits

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Introduction

The number of piglets weaned per female is an important economic indicator used in commercial pig barns. As the litter increases, the cost per piglet decreases (4), but the need for care in this phase increases, requiring greater attention from employees. Improving the maternal ability and performance of piglets in the lactation period could be the key points to reduce the need for handling the litter. To add new traits related to the quality and performance of the piglet in breeding programs requires an accurate knowledge of iteration between all the traits incorporated in its objective and selection index. The aim of this study was estimate genetic correlation between litter performance and litter size traits.

Materials and Methods

Data were obtained from Brazilian nucleus farm on Landrace sow's performance from 2014 to 2018. Data (Table 1) included average daily gain of the litter (ADG), weight produced until weaning without birth weight (WW), number of total born (NTB), number of born alive (NBA), litter size at day 5 (LS5) and pedigree information of all sows. The average number of weaned per sow was 10 piglets.

The genetic correlations were estimated with maximum restricted likelihood, using the software REMLF90 (3). The bivariate trait models include as fixed effect the contemporary group (month-year) and parity order. For ADG and WW were also included as covariates the average birth weight and litter size.

Table 1. Descriptive statistics of the traits.

Trait	Mean	SD[1]	N[2]	Sows[3]
ADG (kg)	0.22	0.05	1698	821
WW (kg)	65.24	17.11	1704	822
NTB	15.97	3.60	2375	951
NBA	14.65	3.27	2364	947
LS5	13.91	1.71	2379	949

[1]Standard deviation; [2]Number of observations; [3]Number of sows.

Results

Both litter performance traits had low phenotypic correlations with litter size traits ranged from -0.04 in WW to 0.04 in ADG (Table 2). Negative genetic correlation of similar magnitude was observed in NSB for ADG and WW. LS5 and NBA shown to be positively related to litter performance traits.

Conclusions and Discussion

The genetic correlations found between traits are weak. The estimated values of genetic and phenotypic correlations between traits are in accordance with those observed in the Landrace breed between WW and NBA (0.07-0.15) by Chen et al. (2). In the Chinese composite dam line, superior and negative genetic correlations were reported between weight and weight gain until d 21 with NTB and NBA (1). Low associations may be due to the fact of the practice of cross-fostering in current production systems (2).

The association found with LS5 demonstrates that there is even more potential for improving the quality of weaned piglets with the addition of a new selection criteria. In addition, the positive genetic correlation, despite presenting low results, is a good indicator that these traits are reflecting on the quality of the piglet, both at birth and at weaning.

Table 2. Genetic (g) and phenotypic (p) correlations of litter performance and litter size traits.

	ADG		WW	
	g	p	g	p
NTB	-0.15	0.02	-0.25	-0.04
NBA	0.16	0.04	0.20	-0.01
LS5	0.12	0.07	0.18	0.01

The correlations found in this study demonstrate that it is possible to use litter performance trait in breeding programs. However, it is still necessary to be careful. There seems to be a complex interaction between ADG and WW with litter size traits, because there are low but negative genetic correlations with NTB. An alternative to minimize a possible negative effect on the litter size is the simultaneous inclusion of a trait related to the number of piglets in the selection index (1).

Acknowledgments

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Genetic Identification of Influenza A Virus' Hemagglutinin in Mexico during 2017- 2019

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Introduction

Influenza A virus (IAV) belongs to the *orthomyxoviridiae* family, causing fever, lethargy, anorexia and nasal secretion in pigs[1]. Losses from IAV infection are estimated at US$3 per pig, but if there are other respiratory pathogens present, they can reach up to US$10 per pig[2]. IAV's RNA virus genome has a 13.6 kb genome. Hemagglutinin (HA) is one of the structural proteins of IAV[3] with a high mutation rate and responsible for the onset of infection at the superficial cells of the respiratory epithelium[4]. The objective of this study was to genetically identify hemagglutinin from IAV variants found in samples from central and northern Mexico during 2017-2019.

Materials and Methods

From October 2017 to June 2019, 1631 oral fluids samples from central and northern Mexico were analyzed to identify IAV; from those, 635 were positive and 996 were negative. Whole genome sequencing was performed on positive samples with cycle threshold (CT) below 28 (43 samples, data in the process of analysis), being the main focus to sequence the hemagglutinin portion of the genome. Sequencing was carried out using the Miseq System equipment and the Nextera-XT DNA kit. Aminoacid-level sequence analysis was performed with CLC Genomics Workbench 10.0.3 software (QIAGEN Redwood City CA). The phylogenetic tree was built applying the Neighbor Joining building model and a 1000 repetitions Bootstrap analysis.

Results

From the 43 samples sequenced, 23 samples corresponded to subtype H1N1, 17 samples to H3N2, and 3 samples to H1N2. In the identity matrix for H1N1, homology of at least 85% is observed among them; at the same time, the study revealed that two H1N1 subgroups were present: the first one with ≥ 96% homology in samples from the states of Jalisco, Guanajuato, Puebla and Veracruz, while the second subgroup shows homology > 94% in samples from Michoacan and Jalisco. The homology in the H1N2 group (samples from the state of Sonora) was ≥ 97% among them. Finally, the H3N2 group (samples from other parts of central and northern Mexico) showed ≥ 88% homology among its sequences. These data imply a high genetic diversity among the H1N1, H1N2 and H3N2 variants. The IAV variants found in this study have already been reported in previous studies[4]. Figure 1 shows the 43 samples' phylogenetic tree.

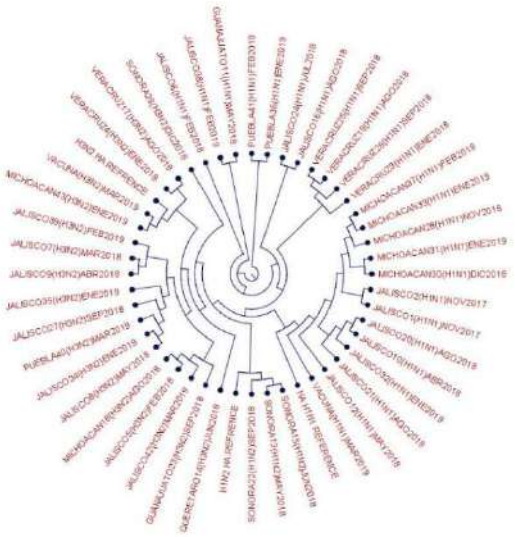

Figure 1. Circular dendogram of IAV hemagglutinin from swine samples collected in central and northern Mexico.

Conclusions and Discussion

Sequencing analysis, along with genotype identification, allows to know the current status of IAV's genetic variants in the different regions of Mexico. This type of information, in addition to that generated when more IAV's whole sequencing results are available, will enhance the understanding of influenza's presentation and dynamics in swine populations. Eventually, all these new pieces of knowledge generated on the disease -in combination with sound principles of biosecurity and animal husbandry- will result on better on-farm control of influeza and, consequently, better production performance.

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Measurements of maternal ability in Landrace sows

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Introduction

Maternal ability can be understood as the female's ability to promote favorable conditions for the development of the piglet. In the first days of life, maternal support is essential, but as the piglet grows, maternal dependency decreases. Traits related to this early development are influenced by a larger maternal component and have an additional complexity (3). Therefore, new measurements of the sow's performance until weaning are necessary in pig breeding programs for improve the quality of the weaned piglet. The aim of this study was to evaluate possible indicator traits of maternal ability in Landrace sows.

Materials and Methods

Data were collected on a Landrace herd between 2014 and 2018 in Brazil. All movements of piglets, weights and sow nurses were recorded. The average number of weaned per sow was 10 piglets. The average daily gain (ADG), weight produced until weaning without birth weight (WW), both in kilograms, and weaning potential (WP), calculated as the number of piglets weaned by the total number of piglets received, were treated as possible measurements of maternal ability (Table 1).

The repeatability model included as a fixed effect month-year of parity, parity order and the covariates of average birth weight and litter size. The analyzes were performed using the gibbs1f90 software (2). A single chain of 400,000 samples was generated with a burning period of 100,000 and a thinning interval of 10.

Table 1. Descriptive statistics of the traits.

Trait	Mean (kg)	SD[1]	N[2]	Sows[3]
ADG	0.22	0.05	1698	821
WW	65.24	17.11	1704	822
WP	0.95	0.08	1452	750

[1]Standard deviation; [2]Number of observations; [3]Number of sows.

Results

Similar heritabilities were observed in ADG, WW and WP (Table 2). In all traits, most of the variation was attributed to the residue. In addition, the effect of permanent environment was greater than the direct additive genetic effect.

Conclusions and Discussion

Heritability estimates were low for all measurements. Values of this magnitude are expected when there is great complexity involving the trait. In addition to the interaction between different genetic materials, traits involving maternal ability in pigs have an additional difficulty related to cross-fostering. This practice makes it difficult to estimate genetic parameters and separate maternal and direct effects (6).

Heritabilities similar to those estimated in this study for traits related to piglet weight (ADG and WW) were reported by Solanes et al. (4) (0.03-0.06) and Stratz et al. (5) (0.03-0.05) in weaning weight.

In WP, although directly related to the number of weaned, the least heritability was observed. Close values (0.02-0.03) were obtained by Hanenberg et al. (1) for the same type of measurement in a population of Landrace. They attribute the underestimation to the tendency that sows with greater maternal ability tend to take care of weaker piglets.

Table 2. Posterior mean estimate of genetic additive variance (σ_a^2), permanent environment variance (σ_{pe}^2), residual variance (σ_e^2) and heritability (h^2) with respective standard error (SE).

Trait	σ_a^2	σ_{pe}^2	σ_e^2	h^2
ADG	0.00003 (0.00)	0.00007 (0.00)	0.00152 (0.00)	0.02 (0.00)
WW	2.82451 (0.01)	4.63021 (0.02)	106.77000 (0.03)	0.02 (0.00)
WP	0.00009 (0.00)	0.00011 (0.00)	0.00561 (0.00)	0.01 (0.00)

Litter growth trait as ADG and WW are directly dependent on the sow's milk production. These measurements can improve the female's nurse capacity and the quality of the piglet. Traits related with weight, despite the low heritability when related with maternal ability, have greater potential to become selection criteria in pig breeding programs because they are routinely collected.

Acknowledgments

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HERD MANAGEMENT

Saccharomyces cerevisiae oral supplementation in piglets challenged with *Mycoplasma hyopneumoniae*: Effect on productive performance and percentage of lung lesions

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Introduction

Enzootic pneumonia (EP) is a swine respiratory disease produced by *Mycoplasma hyopneumoniae (Mh)* and is worldwide distributed. It is cause of important economical losses in porcine industry; therefore, the development of prevention and/or treatments programs is required, mainly with the new restrictions for the use of antibiotics as growth promoters in animal production. The use of yeasts such as *Sacharomyces cerevisiae (S. cerevisiae)* and its derived products have shown positive results in terms of productive performance and immunity of pigs when supplemented in feed diets. Thus, an experiment was conducted to evaluate the effect of *S. cerevisiae* in health and productivity of piglets under a *Mh* challenge.

Material and Methods.

Thirty two newly weaned piglets with an average initial weight of 6.5 kg were randomly assigned to one of 3 treatments: Negative Control (without challenge), Positive Control (Challenge), and Positive Control + *S. cerevisiae* (1.0×10^7 CFU/kg of feed). A diet containing the nutritional requirements for the prestarter phase was offered *ad libitum.* Yeast was added to the piglets diets until week 3 and the challenge with *Mh* was conducted in an isolated aerosol chamber. Blood samples were obtained for detection of antibodies against *Mh*. Animals were monitored daily for typical EP signs. Nasal swab samples were obtained as well for the detection of the *Mh* presence by PCR. Finally, productive performance was evaluated using average daily gain (ADG) as the indicator.

Results

The lungs of pigs in the Negative Control showed a very small incidence of lesions caused by EP (0.4% of the surface in the apical pulmonary lob, while in the lungs obtained from piglets in the Positive Control Group the lesion incidence was greater (14.4%). In the *S. cerevisiae* supplemented group the lung lesion incidence was minimal (3.7%) when compared to Positive Control. PCR test confirmed the presence of *M. hyopneumoniae*; however, *S. cerevisiae* supplemented group was serologically negative to *Mh* antibodies.

The ADG in the Positive Control Group was reduced because of the EP when compared to *S. cerevisiae* Group. While the Negative Control showed the highest ADG during this experiment (Table 1).

Table 1. Final weight of piglets challenged with *Mycoplasma hyopneumoniae*.

Treatment	Neg. Control	Pos. Control	S. cerevisiae
Final weight	22.8 [a]	13.0 [c]	15.2 [b]
Std deviation	0.73	0.39	0.74

[a,b,c] Different letters in the same row means significant difference between the treatments (P<0.05)

Discussion

As expected, *Mh* caused severe lessions at lung level in the piglets challenged in this experiment; however an average reduction of 78% was observed in this matter due to the *Saccharomyces cerevisiae* supplementation in feed when compared to the Positive Control Group. By other side, ADG was severely affected by the EP and the Positive Control Group showed the lowest performance in terms of weight. Supplementation with *S. cerevisiae* seems to reduce the impact of *Mycoplasma hyopneumoniae* on the productive performance of piglets. This effect is probably due to the improvement in gut health caused by the yeast as well as the immunomodulation.

Conclusions

The ADG improvement and the reduction in the lesion score in the *Saccharomyces cerevisiae* supplemented group allowed us to conclude that the dosage of the yeast product supplemented in this experiment was effective to protect the animals after a challenge with *Mycoplasma hyopneumoniae*.

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Palabras Clave: *Mycoplasma hyopneumoniae*, *S. cerevisiae*, Immunomodulation.

Application of an economic calculator to determine the cost of Porcine Reproductive and Respiratory Syndrome at farm-level

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Introduction

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) continues to be a major economic issue for the swine industry worldwide, not only due to acute outbreaks but also endemic infections. PRRS disease severity and consequently financial losses can vary greatly between endemically infected farms. Thus, estimation of damage is challenging and most cost calculations available are general estimates at industry level, derived from anecdotal case reports, or consider just an epidemic period on farm. Therefore, this study aimed to systematically assess the economic effect of PRRS at individual farm-level for endemically infected herds, using a PRRS cost simulation tool (1).

Materials and Methods

In total 21 German sow herds located in different areas were investigated. Inclusion was upon referral by a veterinarian, and criteria were herd size (≥ 100 sows), documented endemic PRRSV infection and no other primary infectious diseases. Data on health and production performance, farm management and environment was collected on each farm, and blood samples taken to confirm its PRRSV status via PCR and ELISA. All data were fed into the cost simulation tool (1), and farm budgets and PRRS costs calculated for all farms. Descriptive statistics (median, range) were then carried out with the resulting values.

Results

In 20 out of 21 herds, PRRSV was detected by PCR, and in all herds, antibodies against PRRSV were detected. The median farm budget across all farms was -31 € per sow and year, compared to a median simulated farm budget of 260 € if farms had been PRRSV negative. The median total loss attributable to PRRSV was 77,000 € (range 17,204 - 322,561 €) per farm per year, corresponding to a median total loss per sow and year of 265 € (range 48 - 586 €). In all cases, the biggest part of the total loss occurred in the fattening part, and the lowest financial impact in the nursery part. With regards to single costs (Figure 1), the biggest loss was seen in the revenue due to lower numbers of fattening pigs sold. In turn, as second highest impact of PRRS, there were remarkable savings in feed due to lower number of animals to be fed. The impact of PRRS on farm profits was -19.1% on average and -41% in the worst case.

Conclusions and Discussion

The results rendered a systematic estimation of average PRRS-attributable losses in endemically infected sow farms, and thereby give a good hint of the PRRS-associated economic damage for the German pig industry. Even in endemically infected farms, farmers face a non-negligible damage and profit from a concerted PRRS control. The calculator has proven itself in the field to render a valid estimation of losses due to PRRS in endemically infected farms.

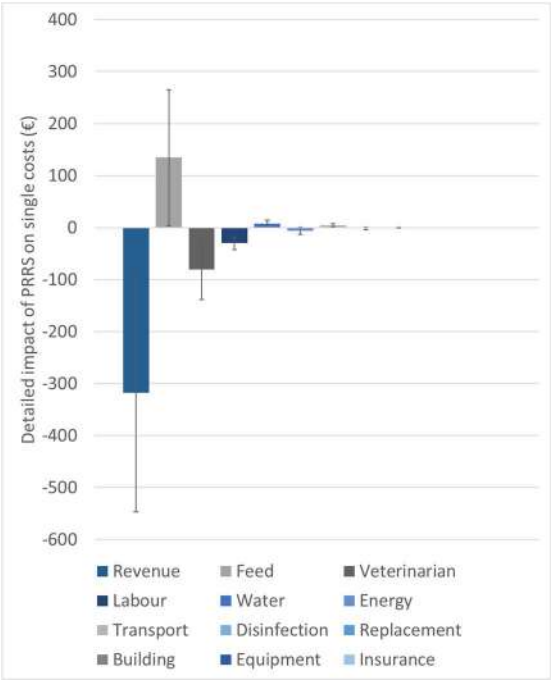

Figure 1. Impact of PRRS on single costs: negative figures indicate losses, positive figures indicate savings.

Acknowledgments

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Reduction of antimicrobial use and resistance in Belgian and Dutch pig farms; i-4-1-Health project results

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Introduction

Reduction of antimicrobial use (AMU) is the first step in curbing antimicrobial resistance (AMR). However, influencing farmer behavior is challenging. In the i-4-1-Health project in the Dutch(NL)-Belgian(BE) cross-border region, 29 pig farms were coached to reduce AMU and study effects on AMR. To assess farmer's attitude and behavior towards AMU the ADKAR coaching tool, scoring for Awareness, Desire, Knowledge , Ability and Reinforcement was adjusted to use for farmers.

Materials and Methods

A pragmatic longitudinal trial was conducted on 29 pig farms for an 18 month period. Farms were included that had high AMU in the year preceding the trial.
Biosecurity level (Biocheck.UGent), technical performance, AMU (treatment incidence/100 days) and AMR were assessed at three farm visits. AMR was determined in *Enterobacteriaceae* from fecal samples (1) (FecalSwab, Copan Italy) on selective agar plates (ChromID ESBL/CARBA/OXA-48, bioMérieux; McC-ciprofloxacin 2 mg/L, in house). Coaching of farmers started four weeks after the first visit, based on a tailor-made action plan. The farms were revisited twice with a six month interval to evaluate implementation and reinforce compliance to the action plan and collect samples.
Mixed effect models with random farm and fixed country and time effects were used.

Results

The initial AMU in weaned pigs was 65% lower for NL versus BE farms and decreased with 53% in BE, and 7% for NL herds after 13 months (Table 1). Biosecurity scores significantly improved in BE farms, but overall were not significantly associated with AMU on farm level. Lowering AMU was significantly associated with higher scores for farmer's Awareness, Desire and Knowledge on AMU and AMR.
Ciprofloxacin-resistant (Cipro-R) and ESBL-producing *Enterobacteriaceae* (ESBL-E) were found on more BE compared to NL farms. No Carbapenem resistance was detected. A significant decrease in Cipro-R was observed over time, but not for ESBL-E. 36% of Cipro-R samples tested ESBL+, in contrast to 16% of Cipro-S samples (adjusted OR=2.4).

Conclusions and Discussion

Coaching towards improvements in infection control and prudent AMU resulted in clear reduction of AMU. The project provided insights in AMR on pig farms.

Table 1. Descriptive results of mean AMU (Treatment Incidence (TI)) and AMR (proportion samples with ESBL producing or Ciprofloxacin resistant *E. Coli*) over time in BE (N=14) and NL (N=15) farms.

Parameter \ Time	T=1	T=2	T=3
TI B farms (%)	45.7	32.8	20.6
TI NL farms (%)	15.9	14.0	14.8
ESBL+ BE farms	12	11	11
ESBL+ BE samples	0.36	0.34	0.40
ESBL+ NL farms	2	1	1
ESBL+ NL samples	0.04	0.01	0.02
CiproR-E BE farms	13	12	13
CiproR-E BE samples	0.33	0.31	0.23
CiproR-E NL farms	2	2	2
CiproR-E NL samples	0.11	0.05	0.05

Acknowledgments

Authors greatly acknowledge participating farmers and the herd veterinarians.
The i-4-1-Health project was financed by the Interreg V Flanders-The Netherlands program, the cross-border cooperation program with financial support from the European Regional Development Fund (ERDF). Additional financial support was received from the Dutch Ministry of Health, Welfare and Sport, the Dutch Ministry of Economic Affairs, the Province of Noord-Brabant, the Belgian Department of Agriculture and Fisheries, the Province of Antwerp and the Province of East-Flanders. Selective and non-selective agar plates, ETEST® strips and VITEK® 2 AST cards were provided by bioMérieux (Marcy l'Etoile, France), FecalSwabs® and tryptic soy broths were provided by Copan Italy (Brescia, Italy). The authors are free to publish the results from the project without interference from the funding bodies, bioMérieux or Copan Italy.

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Evaluation of the Biocheck OppA-ELISA for herd diagnostics of *Haemophilus parasuis*

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Introduction

Haemophilus (H.) parasuis is ubiquitous in swine herds and a member of the normal upper respiratory tract microbiota. Colonization occurs soon after birth and takes place when piglets are still protected by circulating maternal antibodies. *H. parasuis* strains are classified into 15 serovars and many other strains are non-typable. In spite of the variety of strains within a herd, commonly one prevalent strain is associated with an outbreak. Clinical signs are mainly observed in 4- to 8-week-old pigs after stress situations such as transport or mixing into new groups. Acute systemic infection is characterized by a fibrinous or fibrinopurulent polyserositis. *H. parasuis* cannot survive for long, especially at room temperature. Since the cultivation of the pathogen is difficult and often gives unsatisfactory, difficult to interpret results, the indirect detection of several serotypes simultaneously in a single test seems to be a more suitable method for herd monitoring.

Materials and Methods

174 serum samples from six farms with different anamnesis were tested according to the manufacturer`s instructions using OppA-ELISA from BioCheck ER Reeuwijk, Netherlands.

The six farms were characterized as follows:

A. Fattening farm with pleurisy, pericarditis, pneumonia in 10-20% of slaughter lungs
B. Fattening farm with acute deaths (50-80 kg) with detection of serotype 4
C. Fattening farm with lameness in 30 - 50% of fattening pigs in the 2nd half of fattening period
D. Vaccination of piglets for 5th and 7th week of life before sale to the fatteners
E. Vaccination of gilts
F. Randomly selected control-fattening-farm without any clinical problems

With the ELISA test, antibodies against the transmembrane protein oligopeptide permease A can be measured (Macedo et al., 2016). The OppA protein is specific for *H. parasuis* and is produced by different serovars. Antibodies against OppA can only be found in systemically infected pigs but not in animals where only the upper respiratory tract is colonized. Therefore, the detection of these antibodies is suitable to distinguish between only colonized and infected pigs (Macedo et al., 2016). Vaccinated pigs also show antibodies against OppA (Willhelm et al., 2016). The serum titers were compared in order to evaluate the suitability of the ELISA test kit for *H. parasuis* herd diagnostics.

Results

Farm D had the most pigs testing positive (70%), followed by farm C (36.6%) and B (16.6%) and farm A (16.0%) respectively. All pigs from farms E and F tested negative. Only on the vaccination farm D, was the average *H. parasuis* antibody titer (1071 IU) above the manufacturer`s recommended "cut-off" titer of 1000 IU.

Conclusions and Discussion

The results of this study of pigs from 6 different fattening farms demonstrated a typical pattern of infection with *H. parasuis* on the first three farms (A, B and C). In these farms, *H. parasuis* was either detected (farm B, serotype 4) or is suspected to be involved in the existing problems (farm A: polyserositis, pneumonia or farm C: lameness).

Not surprisingly, vaccination farm D had the most seropositive pigs and the highest antibody titers. However, in the study of Galina Pantoja (2014), 90% of the vaccinated sows seroconverted after two vaccinations. These sows were vaccinated significantly later (20 and 28 week of life), compared to the piglets on farm D. Hence, an interaction between maternal antibodies and vaccination antigen, administered in the 5th week of life, cannot be excluded. However, it was surprising that not a single pig tested positive on the second vaccination farm (farm E). It turned out, that after a change of personnel on the farm, the gilts were erroneously no longer vaccinated.

In summary, the OppA ELISA (BioCheck, Smart Veterinary Diagnostics, ER Reeuwijk, Netherlands is a valuable tool for herd diagnostics of HPS, to verify HPS vaccination as well as ruling out the involvement of *H. parasuis* in any herd problems, as long as a sufficiently large sample of sera is examined.

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Improvement of nursery productive performance after PRRS and PCV-2 whole-herd prevention approach in Spain

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Introduction

Porcine Reproductive and Respiratory syndrome (PRRS) costs in Europe are estimated between 100€ and 200€ per sow per year and 5€ to10€ per pig (1).
Porcine circovirus 2 (PCV-2) epidemiology has changed due to the widespread of vaccination (2). The stability of non-vaccinated sow populations can be challenged leading to the production of PCV-2 viremic piglets, as shown in unstable farms, and increasing the infectious pressure in the offspring (3).
Preventing PRRS and PCV-2 related diseases in large production systems is challenging, but is considered as one of the most important drivers for keeping systems producing at target levels with high profitability (4,5).
This is a summary of a field trial designed to evaluate the impact of the whole-herd-approach concept (defined as sows and piglets disease prevention strategy) and the 5 step process (6) on controlling PRRS and PCV-2 related diseases, assessed by nursery improvement performance.

Materials and Methods

The study was conducted in a 1000 sows farrow to wean production system in Spain. At weaning, piglets were placed in 2 all-in-all-out nursery barns. Serum and tissues samples from weaners were qPCR positive for both PRRS and PCV-2. Before the trial sows were vaccinated with another type-1 modified live PRRSv vaccine strain every 4 months.
Whole-herd approach prevention strategy was implemented by immunizing sows with 2 ml of Reprocyc PRRS EU® and 1 ml of Ingelvac CircoFLEX® as shown in table 1.

Table 1. sow vaccination scheme.

	Sow vaccination	
Date (week/year)	Reprocyc PRRS EU®	Ingelvac CircoFLEX®
3/18	X	
7/18	X	
11/18		X
21/18	X	
39/18	X	X
6/19	X	X
26/19	X	

Piglets were vaccinated at weaning (3 weeks of age) with the FLEXcombo® protocol (Circoflex®1ml+ Mycoflex®1ml) from week 3 (2018) and from week 11 with 1ml IM of PRRSFLEX EU® at 17 days of age on regular basis.

For data analysis, a before and after treatment approach, including 3 (31343 pigs) and 4 (22398 pigs) batches respectively, was adopted. The key performance indicators (KPI) compared were final nursery weight, medication cost per pig, mortality rate, economical feed conversion ratio (eFCR), and average daily weight gain (ADWG).
For statistical analysis, Kruskal-Wallis test with Minitab.17.1.0 software (2013 Minitab Inc.) was used and BECAL calculator for economics calculations.

Results

A comparison of the KPIs medians for both periods are depicted in table 2. All the analyzed data numerically improved after de implementation of the whole-herd approach prevention program.
A p-value <0.05 was set up for statistical significance for the differences (Diff.).
The magnitude of the reduction was 52% for mortality and 41 % for medication costs. The improvement of ADWG was 29% and 10% for final weight. Final weight, and the improvement in FCR show a statistical trend

Table 2. Comparison of the KPI averages for the different periods.

	Before	After	Diff.	p-value
Final weight (kg)	18.24	20.02	1.78	0.07
Medic/Pig (€)	2.54	1.51	-1.03	0.15
Mortality rate (%)	7.34	3.49	-3.85	0.03
eFCR (kg/kg)	1.591	1.448	-0.143	0.07
ADWG (grs/day)	240	309	69	0.03

Calculated return on investment (ROI) was **3.19:1**.

Conclusions and Discussion

Disease prevention applied both in sows and piglets is a valuable approach for controlling PRRS and PCV-2 impact in swine herds consistently. The whole-herd vaccination program implemented in this system, had a significant positive impact on nurseries performances and economic data.

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Impact of health status and productivity of sow farms on subsequent wean-to-finish mortality

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Introduction

Sow farm productivity and health status impact the subsequent performance of the weaned cohorts up until market[1, 2, 3]. However, much of the knowledge regarding the association between sow farm performance with downstream efficiency is derived from experimental studies, where many confounders are controlled and the results are limited to the reality of the specific study population, therefore, with limited external validity. The objective of this study was to measure the association between breeding-to-wean (BTW) herds productivity and health, with the subsequent wean-to-finish (W2F) mortality of their progenies throughout the growing phase. This was accomplished based on analysis of aggregated datasets related to the cohort, constructing the flow of the groups from breeding to market, and accounting for single or multiple sources, sites and flows.

Materials and Methods

This research captured reports of cohorts of marketed pigs (closeouts) from June 2018 to July 2019 from a production system in the Midwestern region of the USA. The observational units were the closeouts and the outcome was their respective W2F mortality. SAS software scripts (SAS®, Version 9.4) were developed to connect weekly productivity and health status information from sow farm(s), to the respective closeout information of the weaned pigs composing each cohort. Log-transformation was applied to normalize the outcome distribution. Generalized linear mixed modeling (PROC GLIMMIX) measured the association of the following selected BTW parameters with the W2F mortality of the cohorts: average percentage of pre-weaning mortality (PWM); average weaning age in days (W.age); average number of total pigs born (T.born); average percentage of farrowing rate (F.rate). Each BTW productivity parameter was divided into 4 quartiles. Also, each weaning cohort was tagged with the sow farm health status for porcine reproductive and respiratory syndrome (PRRS) and *Mycoplasma hyopneumoniae.* The health status variables were classified as negative (absence of the pathogen), endemic (presence, with no clinical signs), and epidemic (clinical cases for the disease). For closeouts originated from multiple sow farms, a weighted average was conducted for the productivity BTW variables and, for the health status parameters, the "worst" classification was chosen to follow the cohort through marketing.

Results

The SAS algorithms were able to match and merge data from 1245 closeouts into a single dataset, combining the sow farm health and productivity information of each weaned cohort, to their respective closeout performance report. The W2F mortality geometric mean of the closeouts was 8.6%. Overall, the better it was the sow farm productivity, the lower it was the subsequent W2F mortality (Table 1). Similarly, sow farm health status was also a good predictor of W2F mortality (Table 2). Cohorts originated from PRRS and *M.hyo* epidemic sow farms had the worst W2F mortality. However, no statistical difference was accounted for *M.hyo* despite the numerical difference between groups.

Table 1: Average productivity of the cohorts weaned

Category	Quartiles average			
Average PWM*	10.6%	13.0%	14.6%	17.3%
W2F mortality	7.9%^{a}	8.1%^{a}	8.5%^{a}	10.3%^{b}
Average W.age	15.3	16.7	17.8	20.1
W2F mortality	9.9%^{a}	8.5%^{b}	8.1%^{b}	8.0%^{b}
Average T.born	14.3	14.7	15.1	15.6
W2F mortality	10.0%^{a}	8.9%^{b}	8.3%^{bc}	8.0%^{c}
Average F.rate	78.4%	84.3%	86.7%	90.0%
W2F mortality	10.2%^{a}	8.4%^{bc}	8.8%^{b}	7.9%^{c}

Table 2: Health status of the cohorts weaned

PRRS status*	Negative	Endemic	Epidemic
W2F mortality	7.7%^{a}	8.4%^{b}	13.0%^{c}
M.hyo status	Negative	Endemic	Epidemic
W2F mortality	8.0%^{a}	8.9%^{b}	10.%^{b}

* Different letters between groups represent a statistical difference of W2F mortality.

Conclusions and Discussion

The study revealed a strong association between selected BTW productivity performance and the subsequent wean-to-finish mortality. This study demonstrated the significant role that sow farm performance and health status plays in subsequent W2F mortality, likely due to its association with the quality of the weaned pigs.

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Impact of oral vaccination against *Lawsonia intracellularis* on weight variability at the end of fattening in Iberian pig breed

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Background and objectives

Porcine Proliferative Enteropathy (PPE) caused by Lawsonia intracellularis (L.i.) is an enteric disease of pigs affecting most Spanish farms1. Most pigs are infected subclinically and so this can have a relevant economic impact which has been estimated between 1.3€ and 18.5€ per affected pig2,3. However, most studies compare final batch results and few analyze the impact of vaccination on weight variability. Therefore, the aim of this study was to evaluate the impact of oral vaccination against ileitis on variability of the weight in Iberian breed pigs, reared in an intensive (indoor) production system.

Materials and Methods

This study was conducted in a multi-site farm with 2,500 Iberian sows located in the central area of Spain. Pigs at fattening were suffering from subclinical Ileitis, and L.i. infection was confirmed by ELISA (IgG). A total of 2,880 fattening pigs, i.e. 8 batches of 360 pigs, were included in the study (1,440 vaccinated (V) and 1,440 non-vaccinated (NV) with the nonvirulent live vaccine Enterisol® Ileitis Boehringer Ingelheim Vetmedica GmbH). The fattening units were filled on a weekly basis, and in order to minimize the seasonal impact on results, control and vaccinated batches were filled alternatively. The vaccinated pigs were orally vaccinated 3 weeks after weaning, via drinking water in the nursery unit using Thiosulfate Blue (Boehringer Ingelheim Vetmedica GmbH) as stabilizer. Pigs were raised under the same conditions and housed in pens of 40 pigs, with separation of males and females. All pigs were individually weighed at placement in the fattening unit, 37, 73, 109, 142 and 177 days after. ADG, (kg/d) and antibiotics costs (€) were also assessed.

For the statistical analysis, the Minitab.17.1.0 software (2013 Minitab Inc.) was used.

Results and discussion

The evolution of weight is summarized in figure 1. The weight and SD of weight was the same at placement ($p>0.05$). Nevertheless, the differences between vaccinated and non-vaccinated started just when the seroconversion occurred. The weight of the pigs at 37, 73, 109, 142 and 177 days after replacement were + 1.48, +2.57, +4.64, + 3.2 and + 6 respectively in the vaccinated animals ($p<0.05$).

The SD of the weight at placement was the same. The differences between vaccinated and non-vaccinated animals at 37, 73, 109, 142 and 177 days after placement were -8.26%, -8.76%, -8.7%, -7.96% and -8.7% respectively in the vaccinated animals ($p<0,05$). The ADG, was 657 (V) vs 639 (NV) and the reduction of the use of antibiotics in the vaccinated group represented the 74.6% compared to the non-vaccinated group.

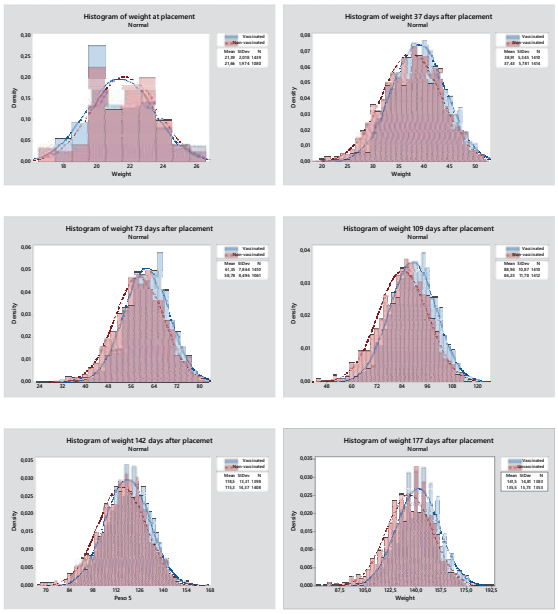

Table 1: Evolution of weight in both groups.

Conclusions

In this field experience, in which pigs were suffering from subclinical ileitis, vaccination with Enterisol® Ileitis increased the weight of pigs and decreased the variability within the vaccinated group compared with the non-vaccinated one, with an undoubted impact on final economic results.

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PRRS type 1 MLV mass vaccination in commercial sow herds is safe

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Introduction

Different PRRS vaccination protocols for sow herds in the Netherlands are used. Off all PRRSV MLV vaccinating sow herds roughly 40% uses whole herd mass vaccination (vaccination of all breeding stock 3-4 times a year) and 50% use a batch vaccination program (vaccination of breeding stock per batch in specific phases in the production cycle) (1). The main arguments not to use mass vaccination protocols are suspected productivity effects due to vaccination during critical phases of the sows' reproductive cycle. In this study we compare production results in sow herds after PRRS type 1 MLV mass vaccination to the baseline production.

Material and Methods

Production data was retrospectively obtained from the farm management systems (AgroVision Netherlands). In total, 8 farms and 70 mass vaccination events using PRRS type 1 MLV vaccines were analyzed. Different registered PRRS type 1 MLV vaccines were used. Following the mass vaccination events we looked into: 1) the pregnancy rate at 35 days after insemination representing return to estrus, 2) farrowing rate, representing total pregnancy losses including abortions, and 3) the number of still born piglets per litter representing piglet quality. The baseline production was defined as the production results in the period up to 6 weeks prior to mass vaccination, the post-vaccination period was defined as the period up to 6 weeks following mass vaccination including the week of mass vaccination.

We performed two types of analysis comparing baseline to post-vaccination production:

- Statistical Process Control (SPC) was used to identify weekly changes of more than 2 Standard Deviations (>2SD) on a herd level. The incidences of >2SD changes over the herds are expressed as average incidences per week.
- Aggregated weekly data from all herds.

Results

Table 1. Incidence rate of negative production results per week of changes >2SD.

Period	Pregnancy rate d35	Farrowing rate	Still born per litter
Baseline production	0,044	0,065	0,048
Post-vacc. production	0,052	0,030	0,032

Table 2. Aggregated production results after mass vaccination events in sow herds compared to baseline production.

Period	Pregnancy rate d35	Farrowing rate	Still born per litter
Baseline production	0.911 ±0.09	0.812 ±0,14	1.39 ±0.67
Post-vacc. production	0.913 ±0.10	0.806 ±0.15	1.38 ±0.59

Discussion and Conclusion

In the aggregated data (table 2) there are no relevant differences in production results. The incidence rate of >2SD changes per week was generally low (table 1) and scattered evenly over the baseline and post vaccination periods (data not shown).

Whole herd vaccination advantages are a larger homogeneity in terms of immunity at population scale (2), better herd immunity, less administration, lower number of missed vaccinations and reduced labor, and is seen as a 'stupid proof' vaccination scheme. Any presumed negative production results like return to estrus and birth of less quality piglets in the weeks following mass vaccinations may be more emotional than rational. Mass vaccination in individual sow farms was experienced with only transient and numerically small changes in productivity when using PRRS type 2 MLV (3) or was found safe when using a PRRS type 1 MLV (4).

We conclude that the PRRS type 1 MLV mass vaccination is safe and recommended for the control of PRRSV in a sow herd.

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Success factors for piglet vaccination

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Introduction

Over the last years in Dutch pig production both the percentage of piglets that are vaccinated and the number of vaccinations per piglet increased. Cost of piglet vaccines per sow per year add significantly to the costs for pig health per farm, up to 75 euro per sow per year and more. Farmers in The Netherlands are allowed to vaccinate the piglets without supervision of a veterinarian, provided that the operator has a vaccination certificate. In a lot of farms the work of vaccinating piglets is seen as unattractive, and is often done by the less experienced staff members. This may lead to more frequently occurring side effects and (partial) vaccine failure. That way not using the full potential that vaccines have and not retrieve the expected return on investment. By interviewing farmers and staff, we tried to have more insight on factors that may influence successful piglet vaccination in the Netherlands.

Material and Methods

In total a number of 50 questionnaires of 11 multi choice questions each were processed during the year 2019. Answers were given by farmers or staff members responsible for piglet vaccination. The results were analyzed using Excel.

Results

The results (n=50) show that:

- 36% of the farms store vaccines in an old refrigerator
- 34% of the farms change the needle used for vaccination at minimal in between every litter or pen
- In 43% of the farms opened and unfinished vaccine vials are stored for later use
- After use the syringes are washed and flushed with tap water without disinfection in 80% of the farms
- Average number of vaccinated piglets per person per hour: 197 (up to 350)
- Average number of vaccination events per batch: 1.8 (up to 4)
- Average cost of labor in the farms per event per vaccinated piglet: euro 0.15 (assuming labor cost per person per hour of 30 euro)

Discussion and Conclusion

Several studies confirm cases in which piglets have been infected by re-using syringes without disinfection after use (1, 2) sometimes leading to the death of the vaccinated piglets. We can only speculate what happens with the piglets that survived the infection. The majority of vaccines used in pig farms have to be kept at 2-8°C, but frequently mistakes are made (3) for example because of old refrigerators that are used like shown in this study.
As mentioned before piglet vaccination is often considered as unattractive work. Creating Good Veterinary Practice conditions will not only lead to better welfare for the piglets, but will also increase the effectiveness of the vaccines used. Apart from vaccine handling according to the Summary of Product Characteristics, a reduction in the number of vaccination events and/ or injections will reduce labor cost and keep motivation of staff at a higher level to properly vaccinate the piglets. Any possible allowed and registered mixing of vaccines contributes to that.

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Factors that relate to low antibiotic use in weaned piglets in the Netherlands

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Introduction

Starting in 2010 the Dutch pig industry has made big progress in the reduction of antibiotic use by applying several approaches (1). In 2018 the reduction was 58% compared to 2009 (the year of reference) (2). Nowadays the highest use of antibiotics – expressed in Daily Defined Dosage Annually (DDDA) - is in the piglets from weaning until about 10 weeks of age (2). Therefore it is useful to focus on the factors that possibly affect the use of antibiotics in this production phase. The goal of this study is to describe risk factors for low antibiotic use in weaned piglets, housed on the same location as the sows, in the Netherlands.

Material and Methods

A list of 18 possible farm specific variables was prepared. During the summer of 2019 veterinarians of De Oosthof veterinary clinic used this list to audit a random selection of 59 individual sow herds. All results were analyzed using Excel by comparing them to DDDA in the weaned piglets in the year 2018, assuming that on these farms no significant changes were implemented during the first 6 months of 2019. 'DDDA high' and 'DDDA low' were defined using the dataset median as cut off value (corresponding with DDDA 11.8). All other numerical variables were categorized the same way: above or below median. Chi^2 testing was performed to find the significant variables that were associated to differences in high or low DDDA.

Results

Significantly associated with low DDDA were:

- Conservative farmers compared to Entrepreneurs ($X^2(0.04,1)$)
- Sow herds smaller than 500 heads (500 being the median value in the dataset) ($X^2(0.0003,1)$)
- Less than 340 piglets per batch (340 being the median value in the dataset) ($X^2(0.005,1)$)
- Not using any of the following vaccinations for piglets: PCV2 ($X^2(0.049,1)$), M.hyopneumoniae ($X^2(0.003,1)$) and PRRSV ($X^2(0.002,1)$)
- The use of 2 or less different vaccinations in piglets ($X^2(<0.0001,1)$)

Discussion and Conclusion

This study is about associations, not about root cause analysis.

As the character of the farmers (Conservative, Caretaker or Entrepreneur) is easily biased by the judgement of the auditor we checked 'herd size not predictive for character' and found that there was a significant association ($X^2(0.002,2)$), making these 2 variables independent of each other.

In general smaller farms align with less piglets per batch and as there was no significant association of DDDA with production rhythm, we assumed 'sow number' and 'piglets per batch' as corresponding variables. It is often mentioned that population size may be a risk for pig health, but the differences between large and small farms are more complicated than just the size of the population (3). In this study, in which there was a lot of variation in DDDA independent of farm size (data not shown), we only address the association found between farm size and DDDA without discussing the multi-variables that come with it.

Previously an association between the use of antimicrobials in weaners and the use of piglet vaccination has been described in Denmark (4). The same association we found in this study. We explain the association by the piglet's health status: in order to reduce production losses in farms that suffer poor piglet health one needs more antibiotics for disease control and may at the same time implement long term interventions like vaccination to prevent disease in piglets, growers and finishing pigs. Poor piglet health causes either economic losses that may motivate the Entrepreneurs or welfare issues that may motivate Caretakers.

All other 11 variables in this study were not found significantly associated with DDDA. We could not define a 'golden bullet' for low antibiotic use.

We conclude that taking the character of individual farmers into account give new opportunities to further decrease weaned piglets DDDA in The Netherlands.

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Iron status and routine for iron supplementation in Swedish piglets

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Introduction

iron deficiency anemia (IDA) is a serious health problem in domestic piglets, resulting in reduced growth rates, increased susceptibility to infectious diseases, and increased mortality rates (1). IDA is measured as the haemoglobin (Hb) concentration in blood. However, the published reference lower cut-off values varies greatly (2), with 90 g/L or 110 g/L being commonly used.

Piglets are born with poor iron reserves. Their daily requirement of 7-16 mg iron is limited by the low content in the sow milk and restricted access to soil as an iron source (3). Thus, routine iron supplementation is recommended to piglets housed indoors, commonly given as an intramuscular (i.m.) injection within the first 3-5 days of life. Alternatively, supplementation with iron paste per os within the first 1-3 days of life may be used, thus avoiding complications such as iron intoxication and local infections following i.m. injections.

Thereafter, a daily supplement of piglet-feed enriched with iron or/and iron products like iron enriched peat, or iron granulate is recommended.

The study aimed to compare the Hb values in 14-day old piglets given an intramuscular injection of iron at 3-5 days of age, to piglets supplemented with iron paste given per os at 1-3 days of age, in both groups followed by daily supplementation of iron-enriched peat. Further, the number of piglets with insufficient Hb concentrations using two different cut-off levels were compared.

Material and Methods

In total, 240 piglets from eight herds were included in the study, four herds being included in each group. The Hb concentration in 14-day-old piglets was measured by a HemoCue Hb 201+ portable device (HemoCue AB, Ängelholm, Sweden) and the results were compared by calculation of the mean of the two groups and by evaluation of the proportion of piglets having values of less than 90 and 110 g/L, respectively.

Results

Piglets receiving a parenteral iron supplementation had significantly (p <0,001) higher Hb levels at 14 days of age as compared to piglets that received iron paste (mean 111.6±14.6 and 91.6±14.0 g/L, respectively) Furthermore, a large proportion of piglets that received iron paste were found to have values <90 g/L (Table 1).

Table 1. Number (n) of piglets with hemoglobin concentrations below 90 and 110 g/L, respectively, in pigs given either of two iron products intended for intramuscular or per oral administration.

Treatment	n	n <90 g/L (%)	n <110 g/L (%)
Iron injection	120	11 (9,2)	50 (41,7)
Iron paste	120	52 (43,3)	108 (90)

Discussion

The results are in agreement with previous results from other studies (4), demonstrating that intramuscular injection of iron is superior to the supplementation of iron paste. Some authors point out the necessity to relate the Hb values to the presence of clinical signs of anemia. However, given the poor growth rate and increased susceptibility to infections described related to low Hb values, it must be equally important to establish a lower cut-off value considering these subclinical signs. In the present study, it was not possible to relate the Hb values to the litter size (data not shown). However, it would be desirable to investigate this further. In addition, it would also be interesting to investigate the Hb values in piglets born and raised outdoors without being supplemented with iron, as well as in wild boar piglets, attempting to establish a “normal” Hb value in 14-day old piglets.

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Risk factors for crushing of piglets in large-scale, commercial pig farms

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Introduction

Pre-weaning mortality of piglets is an important economic and animal welfare issue in swine production worldwide. Crushing by sows is commonly reported as the major cause of these losses (1). Mortality in general, and crushing deaths in particular, are thought to relate to a number of features of the sow and litter as well as various management factors (2).
The aim of the study was to identify environmental and management risk factors associated with piglets crushing during lactation period.

Materials and Methods

This study was carried out at 6 large-scale, commercial pig farms located in the northwest part of Poland. The farms were classified as farrow-to-wean operation with number of sows range from 200 to 8000. A total number of 49,317 litters with 821,334 liveborn piglets were monitored in terms of piglets crushing by sows since Jan 2019 until Dec 2019.
Data about number of piglets crushed were collected for each litter. The presence of potential environmental and management risk factors were reported for each litter.
The simple (univariable) associations between number of piglets crushed for potential risk factors were determined by using chi-square test. Moreover Pearson's correlation was calculated between average monthly outside temperature and percentage of crushes. The significance level was set at $p<0.05$ for all statistical analyses.

Results and Discussion

Average overall preweaning mortality due to crushing (PMC) was 5.03%, ranging from 3.66% to 8.67% depending on the farm.
Furthermore seasonal variation of PMC was observed (Figure 1.). Significant strong positive correlation ($r=0.88$) was found in relation to average monthly outside temperature in the region and PMC ($p<0.0001$). In the summer months (Jun-Sep) average PMC was higher than in the rest of the year. Similar observation was made previously (3, 4). That could be explained by the fact that in hot weather piglets, in order to avoid heat stress, change the place of rest from the space under heat lamp to the slatted part of the floor, which significantly promotes crushing by sows. Moreover, heat stress can also cause alterations in sows, reduce the frequency and duration of nursing periods, increase the time spent urinating or defecating and finally, increase piglet mortality due to crushing (1).
The results of univariable analysis of piglets crushing for the management revealed that higher overall PMC in loose-housed lactating sows than in farrowing crates was observed. Difference was significant ($p<0.0001$) but small 5.27% vs. 4.96%, respectively. Interestingly in 1st week of lactation which is the most important for crushing and 4th week of lactation PMC was lower in loose-housed lactating sows. Factors affecting the increase of PMC in 2nd and 3rd week (perhaps lactation problems in sows) require further analysis.
Surgical castration did not affect PMC in the 1st week of lactation. In other studies negative impact of surgical castration were reported as increase in pre-weaning mortality (5).

Conclusions

From the results of this study we can conclude that seasonal variation of piglets crushing during lactation period is related to hot season of the year. Management factors as loose farrowing systems and surgical castration appears to be risk factor for piglet losses due to crushing, but detailed role of these factors needs further studies.

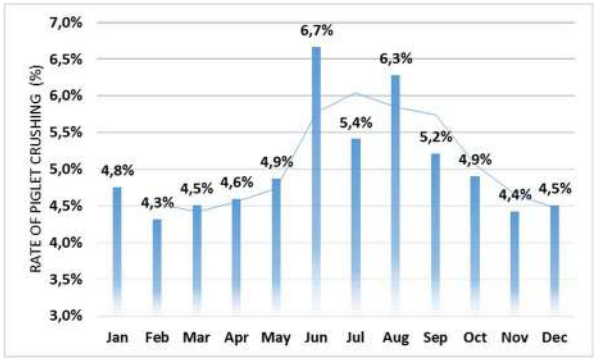

Figure 1. Avarge monthly rate of pigltes died due to crushing in 2019.

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Utility of sampling strategies to monitor the porcine reproductive and respiratory syndrome virus status after an outbreak in a 3000-sow herd in Germany

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Introduction

After outbreaks of porcine reproductive and respiratory syndrome virus (PRRSV) infections in sow herds, the time to stability of the sow herd can take 18 to 55 weeks.[1,2] Different sampling strategies (e.g., processing fluids, serum, or oral fluids) have been described to assess the PRRSV-status after implementing control measures like sow vaccination, improvement of hygiene protocols, lowering stocking density, and piglet flow optimization.[1,3] Thus, we aimed to compare the different described strategies regarding their applicability for monitoring the PRRSV-status in a 3000-sow herd after a PRRS outbreak.

Materials and Methods

In a 3000-sow herd located in Germany, a PRRSV-1 (EU) outbreak occurred and was confirmed by using a PRRSV-1 PCR (EZ-PRRSV MPX 4.0, Tetracore). Subsequently, a hygiene protocol was established, and sows were vaccinated twice in a four-week interval with ReproCyc® PRRS EU. Besides, piglets were vaccinated at three-weeks of age with Ingelvac® PRRSFLEX EU. Five weeks after second sow herd vaccination, every four-weeks processing fluids (4x ten litters of tails and testis), serum (n=30 piglets; pooled 6x five samples), and oral fluids (four cotton ropes of 28 pigs each) specimens were collected from 3-days-old pigs, 3-weeks-old piglets, and 6- and 9-weeks-old pigs, respectively. PRRSV-detection was determined by using a PRRSV-1 PCR and measured again if positive by an in-house PCR to rule out PRRSV-vaccine strain 94881. Results from the different sampling strategies were used to compare the PRRSV-status over time. Data analysis was performed using a non-parametric Kruskal-Wallis test.

Results

Processing fluids and serum samples showed a significant decrease in PRRSV concentrations from week 1 (median: 4.9, range: 4.7 to 6.7) to week 35 (median: 0.0, range: 0.0 to 0.0; p=0.0063) and week 1 (median: 5.3, range: 5.0 to 6.0) to week 35 (median: 0.0, range: 0.0 to 0.0; p=0.0002), respectively. Oral fluids showed no statistically significant differences between the weeks of sampling (p>0.05). A positive correlation was obtained between concentrations in processing fluids and serum (ρ: 0.63 [95% CI: 0.37 to 0.80]; p=0.0001), but not between concentrations in processing fluid and oral fluid or concentrations of serum and oral fluids (both: p>0.05). Results from processing fluids as well as from serum samples revealed an inconsistency in hygiene and pig flow management at the farm, however, an additional integration of an age-dependent pig flow procedure for disease control lead to zero PRRSV concentrations on three consecutive measurements at the farm within 35 weeks after the PRRSV outbreak. According to the variation of measured values for processing fluids and serum at the different time-points, it seems that serum samples had a higher variation compared to processing fluids (Figure 1). During the study period (from week 14 to week 35), a consistent decrease of measured values for processing fluids was observed (Figure 1).

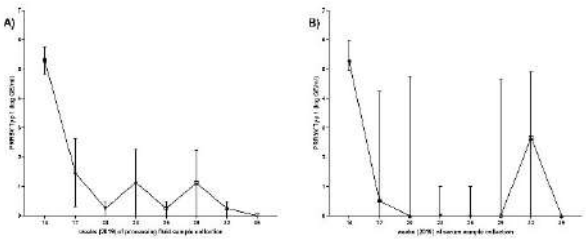

Figure 1: PRRSV concentrations from week 14 to 35 after the PRRSV outbreak in A) the processing fluids (n=40) and B) serum samples (n=30).

Conclusions and Discussion

The 3000-sow herd reached the time to stability of the sow herd within 35 weeks after the detected PRRSV outbreak. The implementation of a strict age-dependent pig flow lead to zero PRRSV results on three consecutive measurements. Processing fluids and serum samples were equivalent sampling strategies to monitor the PRRSV-status regarding long-term evaluation, whereas processing fluids showed more consistent values regarding data interpretation of single examination days.

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Influence of the concentration of IgG at weaning on the post weaning period

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Introduction

The immunocrit assay (1) is a useful tool to measure maternal IgG uptake in serum of new born piglets. However, the immunocrit value at weaning and the correlation with period after weaning are relatively unknown. The objective of this study is to measure the total IgG after weaning and whether this is correlated with zoo technical performance.

Materials and Methods

On a commercial sow farm, piglets were weaned at the age of 28 days. Littermates were kept together in a single pen after weaning. Per pen 4 pigs were selected and were weighed at weaning, 24 h after weaning and at the age of 10 weeks. Blood was collected 24h after weaning and at the age of 10 weeks. Total serum IgG was measured using the immunocrit assay.

Results

Forty pigs were weaned at the age of 28 days. Two pigs died after weaning and two pigs were relocated within the farm. In total 36 piglets could be followed until the age of 10 weeks. Total IgG rose from 16.5 mg/ml 24 h after weaning to 21.5 mg/ml at 10 weeks of age, indicating antibody production by the weaned pig's immune system during the post weaning period. Descriptive statistics of the collected data are presented in Table 1.

Table 1 Descriptive statistics of the collected data at weaning (W.), 24 hours after weaning (24h aW) and at 10 weeks of age (10w).

	Weight (kg)			IgG (mg/ml)		
	WW	*24h aW*	*10w*	*24h aW*	*10w*	*ADG g/d*
Mean	7.34	7.49	24.9	16.5	21.5	417
Median	6.94	7.23	24.9	15.4	20.7	417
St. dev.	1.21	1.32	3.25	9.3	6.0	58
Min	5.76	5.53	18.6	1.2	10.7	303
Max	10.73	10.99	33.7	39.7	40.5	571
n	40	40	36	40	36	36

Data was analyzed with (multiple) linear regression to predict the ADG (y) in the post weaning period. One outlier was excluded (24haW of 2.8 kg), remaining 35 piglets in the regression analysis. Weaning weight ($r = 0.54$, $R^2 = 0.30$ Fig.1), 24h weight gain after weaning (WG24; $r = 0.37$, $R^2=0.13$) and IgG 24haW ($r=0.37$, $R^2= 0.13$; Fig.2) were significant correlated with ADG after weaning. The combination of IgG and weaning weight resulted in a significant model for the prediction of ADG post weaning (ADG = 194.9 + 2.17*IgG + 24.67*Weaning weight + 52.67*WG24; $R^2 = 0.51$, $p<0.001$).

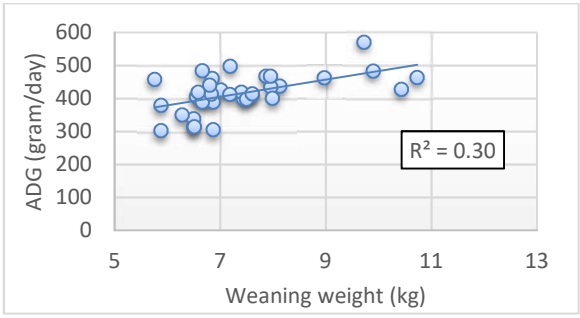

Figure 1. Correlation between weaning weight and post weaning ADG ($p<0.0001$).

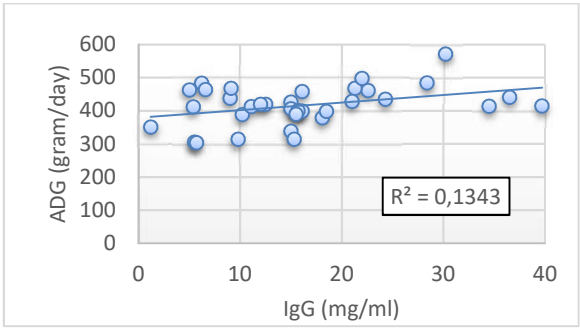

Figure 2. Correlation between IgG 24h after weaning and post weaning ADG ($p=0.03$)

Conclusions and Discussion

The model explains for 51% the variation in ADG post weaning. The correlations of weaning weight and IgG around weaning might be confounded with the IgG at birth and birth weight. The birth weights and IC in the newborn piglets are unfortunately unknown for the piglets in this study. However, weaning weight and IC24aW are not correlated ($r = 0.02$ $p = 0.89$) and therefore do not indicate a strong correlation between these. Even so WG24 and WW were not correlated with each other ($r = 0.13$; $p = 0.42$).

It can be concluded that WW, WG24 and IgG at weaning are important parameters correlated with the ADG post weaning. This demonstrates the importance of a good and fast colostrum uptake directly after birth, high weaning weights and a high feed intake during the 24h after weaning, resulting in positive effects on ADG during the post-weaning period.

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Danish farmers reduce gastric ulcers in sows

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Introduction

For the last decade, gastric ulcers have been of great concern in sows in Denmark, and studies have shown a decrease in sows with severe ulcers from 25% in 2011 to 9% in 2017-2019[1, 2]. One of the main risk factors for development of gastric ulcers is small particle feed[3]. However, small particle feed gives better feed economy and enables sows to consume enough feed to cover the energy need for milk production[4]. Infections has also shown to affect the risk of developing gastric ulcers [5]. However, a lot of factors predispose for development of gastric ulcers and therefore the actions taken to prevent or treat gastric ulcers can be many. Thus, this study aimed to investigate the actions taken by the Danish farmers to reduce the prevalence of gastric ulcers in sows.

Materials and Methods

This study is part of a large national screening for gastric ulcers in sows in Denmark. From 2017 to 2019, 1,037 sow herds have been screened for gastric ulcers in sows. From each herd, 20 stomachs were removed at slaughter and investigated by pathologists. The stomachs were scored on a scale from 0-10, with 0 being no ulcer and 10 the most critical. Severe ulcers were defined as score 8-10. However, defining a herd problem, a herd should have more than 10 of the 20 (>50%) stomachs given scores 7-10. In these cases, the herd manager or owner should produce an action plan to reduce gastric ulcers in the herd. The action plans were collected, either by receiving the written action plan or by brief interviews with the farm manager or owner.

Results

Out of the 1,037 investigated sow herds, 34 should create action plans. All 34 herd managers or owners were contacted by phone and for 22 herds the responsible person was reached. Out of those 7 would not share the action plan and 8 had no written action plan or had not created it yet, but were interviewed with regards to actions taken or planned. A written action plan was received from 7 herds.

The most frequent action described in the written action plans was to increase the particle size in the feed (n=6). Addition of 10-15% rolled barley was done in 3 herds, one herd provided extra straw and one herd added special gut health improving feed additives to the feed. Two herds had made a follow-up investigation of the gastric health, which showed an improved gut health in the herds. One herd had not yet performed such an investigation but is going to after implementing the actions.

In the cases where only an interview was performed, the herd managers/owners explained that they had, or will, increase the particle size of the feed, and a few had increased the fiber fraction in the feed by adding sugar beet pellets and/or providing straw to the sows.

In several of the herds, more than one action was taken to reduce gastric ulcers.

Conclusions and Discussion

In most cases, increasing particle size of the feed was done to reduce gastric ulcers in the included herds. Furthermore, rolled barley and addition of fiber was an often-taken action. These findings correspond well with the general recommendations for reducing the risk of gastric ulcers. Also, since 2011, there is a tendency for the feed producing companies to enhance the particle size in sow feed, and the authorities have had great focus on supplement of straw to sows, which has also shown to be useful in reducing gastric ulcers[6].

In none of the herds, actions toward diseases were taken, even though some studies have found a correlation between diseases and gastric ulcers. This could be because of a general high health status in the farms and therefore first choice actions were taken other ways.

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Purchasing policy, quarantine and acclimation practices of breeding gilts in pig farms

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Introduction

Purchasing of breeding gilts is common practice, but it is a risky event in terms of biosecurity. The frequency of purchase and the number of animals purchased increase the risk for pathogen transmission between farms. To limit this risk, it is important to respect a good quarantine period (1,2). During the quarantine period pigs can be observed for presence of clinical signs and/or tested for the presence of pathogens. Additionally, they can be vaccinated to protect them against pathogens circulating on the farm (3,4). The present study investigated the introduction procedures of gilts in Belgian pig farms as a first step to optimize the management of purchasing breeding gilts.

Materials and Methods

A questionnaire with 20 questions related to gilt introduction was designed by the main authors, partly based on some questions from the risk-based biosecurity scoring tool Biocheck.UGent (www.biocheck.ugent.be). The questionnaire was divided into three parts: purchasing policy, quarantine period and acclimation practices of gilts. Belgian farmers were contacted and visited by researchers of Ghent University, veterinarians at Animal Health Care Flanders and pig practitioners.

Results

Sixty-three Belgian farms completed the questionnaire. Most farms (83 %) used batch management systems, with the 3-week and the 4-week system being equally popular (32 % of the cases each).

Following tables show a description of the continuous (Table 1) and categorical variables (Table 2) of the questionnaire.

Table 1. Description of continuous variables

Variable	median	range
Sows in the herd	300	85 - 2500
Frequency of purchase (times per year)	6	1 - 13
Age at purchasing (weeks)	24	9 - 37
Duration of quarantine (days)	42	14 - 140
Vaccination against different pathogens	7	2 - 12

Table 2. Description of categorical variables

Variable	n	%
Purchasing of breeding gilts		
Yes	35	56
No	28	44
Presence of a quarantine unit		
Yes	33	94
No	2	6
Location of the quarantine unit		
External – followed by internal quarantine	1	3
External – adding gilts immediately to herd	0	0
Internal – isolated stable	19	58
Internal – separate unit within a stable	13	39
Internal – together with other pigs on the farm	0	0
Monitoring for disease[1]		
Yes, for Brachyspira	3	5
Yes, for other pathogens than Brachyspira	9	14
No	53	84
Vaccinations[1,2]		
PRRSV	54	86
Parvovirus	60	95
Erysipelas	59	94
Acclimation practices[1]		
Cull sows	11	17
Afterbirth	4	6
Feces from suckling piglets	11	17
Feces from weaned piglets	2	3
Diarrhea from piglets	1	2
Other	18	29
No	27	43

[1]Farmers could give several answers to this question, therefore the sum of the percentages can exceed 100 %. [2]In this table only the three most common pathogens are mentioned.

Conclusions and Discussion

Fifty-six percent of the farms purchase breeding gilts, however there is a lot of variation in frequency of purchase and the age of purchased breeding gilts.

On 94 % of those farms a quarantine unit is used, where on most farms the quarantine is located on the farm itself. In general the gilts are kept in the quarantine for six weeks, which is a long enough period to observe them for clinical symptoms caused by different pathogens and to apply acclimation practices before introducing them in the sow herd.

The most common acclimation practice is vaccination with a commercial vaccine (using different schemes). Giving feces from suckling piglets and contact with cull sows is used as well to expose the gilts to farm-specific pathogens.

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Time spent by farm staff in different zones of a pig farm

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Introduction

Proper management of a pig farm is crucial for animal health and welfare as well as for optimal performance. Farmers should spend sufficient time in the stables with their animals, especially on important days such as farrowing, estrus stimulation and artificial insemination. However, there is no scientific data on the amount of time employees spend in the stables, neither on the variation depending on the type of activities.

The system B-eSecure has been developed mainly to explore movements of farm staff within a farm. The system also allows quantifying the time spent by farm staff in the different farm zones (1). The present study, which was part of a larger research project on biosecurity in pig farms, quantified the time spent by farm staff in different stables of a commercial farm without specific health problems.

Materials and Methods

This study was conducted on a pig herd with 300 sows practicing a 4-week batch management system (weaning at 3 weeks of age). All weaned piglets (1450) and part of the fattening pigs (480) remained on the farm. Pre-weaning piglet mortality was 12 %, the number of weaned pigs per sow per year 31 and the pregnancy rate was 91 %. Automatic feeding was used for all age groups.

Farm staff had to wear a small device (beacon). This sent a Bluetooth signal to the B-eSecure devices, which were installed in the farrowing, nursery, fattening, gestation and quarantine unit. These devices transferred data via Wi-Fi to an online platform. Data were collected from October 2019 until February 2020, covering a cycle of 4 successive sow batches. As the farm practiced a batch management system, the main activities were clustered on specific days.

Results and Discussion

Table 1 shows the time spent by all employees in the different farm zones and concerning specific activities. The following activities in each batch were considered: sow farrowing (3 days), routine interventions of piglets (day 2-7 after farrowing) (tail docking, castration, ear tagging, iron injection, treatment against coccidiosis, *M. hyopneumoniae* vaccination), piglet vaccination against porcine circovirus type 2 (day 16), weaning (day 21), estrus stimulation (day 1-4 after weaning) and insemination of sows (day 4-7 after weaning).

During the entire period, the work in the stables was on average 7.3 person-hours per day. As expected, most of the time was spent in the farrowing unit, followed by the nursery and the gestation unit. Logically, least time was spent in the quarantine unit as there are only a limited number of animals and the work mainly implies daily supervision and implementing adaptation practices (*e.g.* vaccinations). The average time spent in the fattening unit was also low, first because only part of the weaned pigs were fattened on the farm, and second because no specific interventions apart from daily supervision should be done. On days of specific activities such as piglet vaccination, weaning, and estrus stimulation, the time spent in the stables was significantly higher. The number of person-hours per day during farrowing (3d) and routine interventions for piglets (5d) were only slightly higher than the average likely because these activities were spread over different days.

Table 1. Time spent (person-hours/day) in different farm zones and in relation to activity

	Farm zone (… unit)					
Activity	Farr	Nurs	Fatt	Gest	Quar	Total
A	3.2	1.8	0.4	0.6	0.2	6.2
B	4.9	1.9	0.5	0.5	0.3	8.1
C	6.0	2.5	0.5	0.5	0.5	10.0
D	4.9	2.8	0.3	2.6	0.1	10.7
E	2.3	1.6	0.5	6.7	0.2	11.3
F	1.5	1.6	0.4	4.4	0.1	8.0
Other	1.5	2.2	0.6	0.9	0.3	5.5
Entire period (%)	2.7 (37)	2.1 (29)	0.5 (7)	1.7 (23)	0.3 (4)	7.3 (100)

Farm zones: Farrowing, Nursery, Fattening, Gestation, Quarantine. Activities: A farrowing (3d), B routine piglet interventions (5d), C PCV2 vaccination (1d), D weaning (1d), E estrus stimulation (4d), F insemination (4d), Other (12d), Entire period (4 times 28d = 112 days)

Conclusions

The results showed significant variation in time spent by farm staff depending on the farm zone and specific activities. The overall person-hours/day in the stables was low, suggesting that the staff worked efficiently. The registered time is not equal to total labor time, as work outside the stables is not included.

Acknowledgments

PigCHAMP pro Europa, MSD Animal Health

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Reproductive performance monitored with statistical process control over 2 years in a farm, which faced an acute PRRS outbreak and implemented PRRS vaccination

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Introduction

Infection with porcine reproductive and respiratory syndrome virus (PRRSV) may lead to significant losses in productivity of breeding and growing pig herds.[1,2] Performance data was monitored over two years in a commercial farm in Croatia, which faced an acute PRRS outbreak and subsequently implemented vaccination against PRRSV. The present abstract presents the results of sow performance. Nursery mortality is presented in a second abstract.

Materials and Methods

The study was conducted on a one site, farrow-to-finish farm with 2000 sows in Croatia. The farm is PRRSV positive since 2014, however, the first introduction never caused noticeable changes in performance and production was stable. A new, acute and severe PRRS outbreak occurred in late 2018, which negatively influenced reproduction (from week 44/2018) and shortly after the growing pig performance (from week 48/2018).

No PRRS vaccination was implemented prior to this new outbreak. A PRRS control program started early 2019. The herd was loaded with gilts and closed for six month. Management changes implemented to reduce PRRSV transmission included limited cross-fostering, no use of foster sows (until Sept. 2019), change of needles and disinfection of surgical blades between litters. From week 8/2019 the breeding herd, including all gilts, was vaccinated twice four weeks apart with ReproCyc PRRS EU®, followed by routine mass vaccination every three month. Vaccination against PRRSV was also implemented for piglets.

Four key performance parameters were analyzed via statistical process control (SPC) starting from week 20/2018 until week 20/2020. Weekly data was compared between three different periods, respecting the phases before (period 1), during the acute PRRS outbreak (period 2) and after the implementation of PRRS vaccination (period 3). One other major event was reported for the observational period. From week 25/2019 a remarkable drop in the pregnancy rate was noted that continued over several weeks (event 1). A full diagnostic work-up revealed spermicide substances in the tubes used for semen storage. The problem was solved by the exchange of the respective materials. Consequences of event 1 were noticeable in several other parameters following through the production line. No other changes of vaccinations in sows or major changes in feeding or housing were implemented during the observational period. A transition period, during which results could not be assigned to one or the other treatment, as well as results influenced by event 1 were excluded.

Results and Discussion

Table 1 presents the detailed results of the reproductive parameters.

Table 1: Mean and standard deviation of 4 reproductive parameters during 3 comparison periods. Different superscripts within the rows indicate significant differences ($p<0.05$).

	Period 1	Period 2	Period 3
Pregnancy rate	93.4±0.7^a	92.9±0.7^a	93.9±0.3^a
Farrowing rate	91.9±0.5^a	85.0±1.9^b	89.9±0.4^c
Live-born piglets	14.6±0.1^a	13.5±0.2^b	14.2±0.1^c
Pre-weaning mortality	10.7±0.4^a	19.2±1.7^b	12.7±0.6^c

Reproductive performance was stabilized after implementation of the PRRS control program following the acute PRRS outbreak. Results in period 3 (3.1 and 3.2 combined) were close to pre-outbreak values and variation (i.e. standard deviation) could be stabilized at pre-outbreak levels ($p=0.601$) leading to more predictable production (table 1; figure 1).

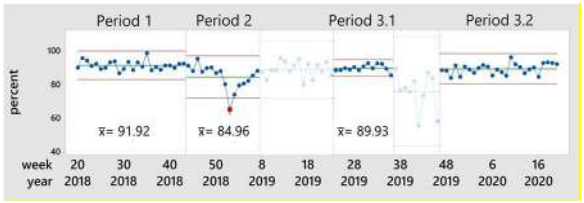

Figure 1. Farrowing rate (%) in the different periods presented in an I-MR chart. The mean is calculated for each period ($\bar{x}$; 3.1 and 3.2 combined) and indicated by the green line. Red lines indicate the upper and lower control limits. Data during transition period and event 1 are blended out.

Conclusions

Vaccination and biosecurity measures are valid tools to stabilize production after an acute PRRS outbreak. Statistical process control offers a straightforward way to analyze large continuous data sets, as in the present case, taking into consideration mean and variation of the data associated with process changes.

References

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Nursery mortality monitored with statistical process control over 2 years in a farm, which faced an acute PRRS outbreak and implemented changes in the vaccination program

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Introduction

Infection with porcine reproductive and respiratory syndrome virus (PRRSV) may lead to significant losses in productivity of breeding and growing pig herds.[1,2] Performance data was monitored over two years in a commercial farm in Croatia, which faced an acute PRRS outbreak and subsequently implemented changes in the vaccination program. The present abstract presents the results of nursery mortality. Sow performance is presented in a second abstract.

Materials and Methods

The study was conducted on a one site, farrow-to-finish farm with 2000 sows in Croatia. The farm is PRRSV positive since 2014, however, the first introduction never caused noticeable changes in performance and production was stable. A new, acute and severe PRRS outbreak occurred in late 2018, which negatively influenced reproduction (from week 44/2018) and shortly after the growing pig performance (from week 48/2018).

Piglets were vaccinated against porcine Circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* (Mhyo). No PRRS vaccination was implemented prior to this new outbreak. A PRRS control program was initiated early 2019. The herd was loaded with gilts and closed for six month. In week 8/2019 vaccination against PRRSV was initiated. The breeding herd, including all gilts, was vaccinated twice four weeks apart with ReproCyc PRRS EU®, followed by routine mass vaccination every three month. Concurrently with the first mass vaccination of the breeding herd, all piglets in the farrowing unit at the age of 14 days and older were vaccinated with Ingelvac PRRSFLEX® EU, followed by routine vaccination at three weeks of age. Piglets were concurrently vaccinated against PCV2 and Mhyo. Starting with week 39/2019, previous PCV2/Mhyo vaccines were replaced by FLEXcombo®. No other major changes of vaccination, management, feeding or housing were implemented during the observational period.

Mortality in nursery was analyzed via statistical process control (SPC) starting from week 20/2018 until week 20/2020. Weekly data was compared for four different periods, respecting the different vaccination protocols and the phase of the acute PRRS outbreak until the start of the PRRS control program. Transition periods, during which results could not be assigned to one or the other treatment, were excluded.

Results and Discussion

Figure 1 presents the data over the period of two years, split in four different comparison periods, and two transition periods.

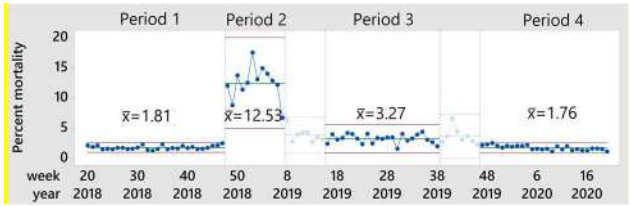

Figure 1: Nursery mortality (%) in the different periods presented in an I-MR chart. Period 1: PCV2/Mhyo vaccination; Period 2: acute PRRS outbreak; Period 3: PRRSFLEX + PCV2/M.hyo; Period 4: PRRSFLEX + FLEXcombo. The mean is calculated for each period ($\bar{x}$) and indicated by the green line. Red lines indicate the upper and lower control limits. Transition periods are blended out.

A significant increase of nursery mortality ($p<0.001$) was noticed along with the acute PRRS outbreak. After the start of the PRRS vaccination program mortality dropped significantly ($p<0.001$), but did not reach the pre-outbreak level ($p<0.001$). After additional change of vaccines against PCV2 and Mhyo, the mean and the standard deviation of nursery mortality dropped again significantly ($p\leq0.003$) to the pre-outbreak level ($p=0.619$).

Conclusions

Vaccination is a key measure to prevent and control diseases in modern swine production. Three major pathogens in pig production, PRRSV, PCV2 and Mhyo, do influence each other and may all be controlled, but not eliminated by vaccination.[3] Consequently, as presented, changes in the vaccination program and/or individual vaccines within the program may change the overall disease situation and the associated performance. Statistical process control is a valuable tool to analyze large continues data sets, taking into consideration mean and variation of the data associated with process changes.

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Impact of sow herd vaccination against Porcine Reproductive and Respiratory Syndrome Virus type 1 (PRRSV-1) on reproductive performance on a one-site farm in Serbia

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Introduction

PRRSV can cause significant impact on reproductive performance in endemically infected herds.[1,2] This study aimed to investigate sow performance before and after implementation of a sow herd vaccination against PRRSV on a farm in Serbia.

Materials and Methods

The study was conducted on a farrow-to-finish farm with 2300 sows producing with weekly batch farrowing. The producer was dissatisfied with low farrowing rate with high variation ($\bar{x}$±SD=77.8%±3.4). Diagnostics confirmed the presence of PRRSV. No PRRS vaccination was implemented prior to this study.
Vaccination started in October 2018 with double mass vaccination (four weeks apart) of the breeding herd, including gilts over 150 days of age, with ReproCyc PRRS EU®. Vaccination of the sow herd was repeated every three months. Performance data of sows inseminated from October 2018 onwards was collected over a period of 7 months after the start of the vaccination program (last insemination April 2019, last farrowing August 2019) and compared with the same period one year before. No other changes in vaccination programs or major changes in management, feeding or housing were implemented during the observational period.

Results and Discussion

PRRS vaccinated sows (vac) had a significantly higher pregnancy rate ($\bar{x}_{vac}$=92% vs. $\bar{x}$=87.9%, p=0.023) and farrowing rate (figure 1, p=0.023) and significantly lower returns to estrus (figure 2, p=0.007).

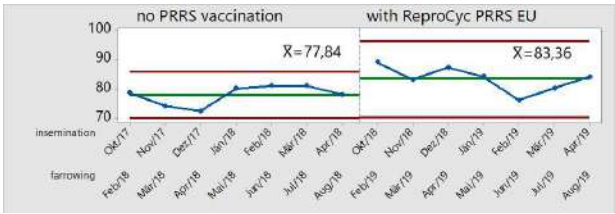

Figure 1. Farrowing rate in percent before and after implementation of PRRS vaccination presented in an I-MR chart. The mean is indicated by the green line, the upper and lower control limits by the red lines.

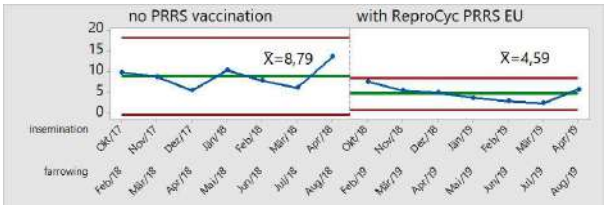

Figure 2. Return to estrus rate in percent before and after implementation of PRRS vaccination presented in an I-MR chart. The mean is indicated by the green line, the upper and lower control limits by the red lines.

Live-born piglets per litter did not differ significantly before and after the implementation of PRRS vaccination ($\bar{x}_{vac}$=10.4 vs. $\bar{x}$=10.2, p=0.303).
The results show a positive impact of sow herd vaccination against PRRSV on the performance of the sows. Based on the farrowing rate and the number of live-born piglets only the farm produced 2979 live-born piglets extra.

Conclusions

In relation to the cost of sow herd vaccination, every extra piglet has to be worth at least € 4.60 only to equal out the investment.

References

1. Zimmerman et al. 2019: Diseases of Swine, 11th edition: 685-708.
2. Nathues et al. 2017: Preventive Veterinary Medicine 142: 16-29.

Effect of Optimizing the Sow Herd Vaccination Program on a Farm in Taiwan Infected with the Pseudorabies Virus

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Introduction

Pseudorabies (PR) is still considered a major swine disease in Taiwan and the majority of Taiwanese farms use live gE-deleted PR vaccines in breeding herds and fattening pigs as a routine vaccination programme. The aim of this study was to evaluate the effect of an optimized vaccination programme with a combination of commercial vaccines on a gE-negative sow herd in Taiwan.

Materials and Methods

The trial was conducted on a PRV-infected commercial farrow-to-finish farm in Taiwan from May 2018 to June 2019. The PR status was determined before and after the optimized programme by serology of gE and gB antibodies using commercial ELISA kits (BioCheck, The Netherlands). The original immunization programme before conducting this trial was vaccination of gilts with a live gE-deleted PR vaccine only (baseline). During this study, the basic vaccination programme for the breeding herd was mass vaccination with a live gE-deleted PR vaccine (HIPRASUIS® AD) every 4 months (group 1); and the optimized programme added an inactivated PR vaccine (HIPRASUIS® AD BK), 4 weeks before farrowing (group 2) (Table 1). Serum samples were collected from the sow herd, and from their offspring at 3, 9 and 12 weeks of age. Differences between groups were tested by Kruskal-Wallis test. All the statistics were performed with R software.

Table 1. PRV immunization programmes

	Gilts	Breeding herd
Baseline	2 shots before mating with live gE-deleted PR vaccine	No vaccination
Group 1 (basic)	2 shots before mating with live gE-deleted PR vaccine[a]	Mass vaccination every 4 months with live gE-deleted PR vaccine[a]
Group 2 (optimized)	2 shots before mating with live gE-deleted PR vaccine[a]	Mass vaccination every 4 months with live gE; Inactivated PR vaccine[b] 4 weeks before farrowing

[a]: HIPRASUIS® AD BK; [b]: HIPRASUIS® AD BK

Results

The serological screening for gE antibodies showed that the sow herd, at weaning and 9 weeks of age was negative. At 12 weeks of age 10% of fatteners were gE-positive (infected with the field virus) in the baseline group while group 1 and group 2 remained negative.

Mean PRV gB ELISA S/P ratios are shown in figure 1. Group 2, with an optimized immunization programme (adding an inactivated vaccine), showed a higher level of antibodies in comparison to the basic programme (group 1, $p<0.001$) and baseline group ($p<0.001$).

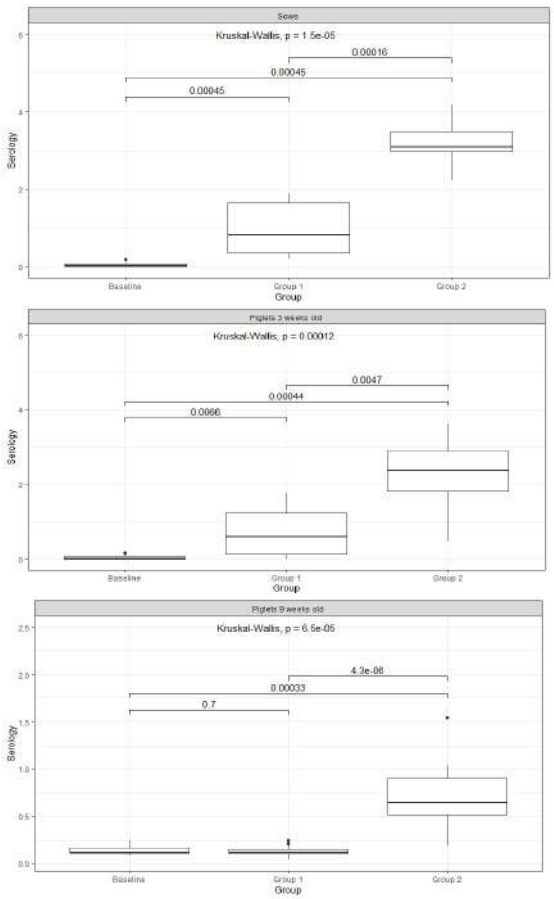

Figure 1. Mean PRV gB ELISA S/P ratios.

Conclusions and Discussion

The optimized vaccination programme for PR using a live gE-deleted PR vaccine (HIPRASUIS® AD) for breeding herd mass vaccination (3 times/year), adding an inactivated PR vaccine (HIPRASUIS® AD BK), 4 weeks before farrowing represent the highest antibody response. This level of protection could increase the duration of maternal antibodies in the offspring and may be a good way to postpone PRV infection and/or vaccination timing in growers.

References

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Impact of fecal consistency score on feed conversion and daily weight gain in nursery pigs

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Introduction

Fecal consistency scoring is used to assess gut health and diagnose enteritis in nursery pigs. However, the relationship between fecal consistency and performance in terms of growth rate and feed efficiency remains to be explored. Particle size in feed is known to affect gut health, daily weight gain and feed conversion ratio.

The objective of the present study was to investigate the association between fecal consistency, feed conversion ratio and daily weight gain. The association was studied in two groups of pigs fed a finely ground pelleted feed or a coarsely ground meal feed.

Materials and Methods

A total of 108 pens with 1080 nursery pigs in the weight interval from 7 to 30 kg were included in the experiment.
All pigs received feed with similar ingredients, but half received a coarsely ground meal feed while the other half received a finely ground and pelleted feed. Consumption of feed was recorded pen wise and measured in Danish Feed Units for grower pigs (FUgp) (1). One FUgp corresponds to 7.38 MJ potential physiological energy. Average feed conversion ratio (AFCR) per kg body weight gain (BWG) per pen was calculated.
The consistency of feces was scored at pen level three times a week using a score where: 1 = normal, 2 = mushy, 3 = watery. Average fecal consistency score (AFCS) for pens with score 2 or 3 were adjusted by subtracting the proportion (1/3 or 2/3) of fecal pools that didn't reach the score.
Fecal floor samples were tested for presence of intestinal pathogens by PCR at the end of the experiment (2). Number of antibiotic treatment days was recorded per pig. Statistical models included type of feed (meal or pellets) and room as explanatory variable, with AFCS and batch as fixed and random effect, respectively. Response variables were AFCR and ADG.

Results

Fecal consistency (AFCS) was strongly associated with feed conversion ratio and average daily gain (fig 1.) The effect of 0.1 increase in AFCS was 0.043 FUgp/kg BWG and -18 g ADG, ($P<0.005$). Very few pens had watery feces. AFCS was 1.14 for meal fed pigs and 1.18 for pigs fed pellets. Average antibiotic treatment days per pig were 2.26 and 3.13, respectively. *L. intracellularis* was detected in moderate levels. ADG was increased (440 vs. 340 g/day) and AFCR improved (1.84 vs. 2.21 FUgp/kg) in pelleted compared to meal fed pigs.

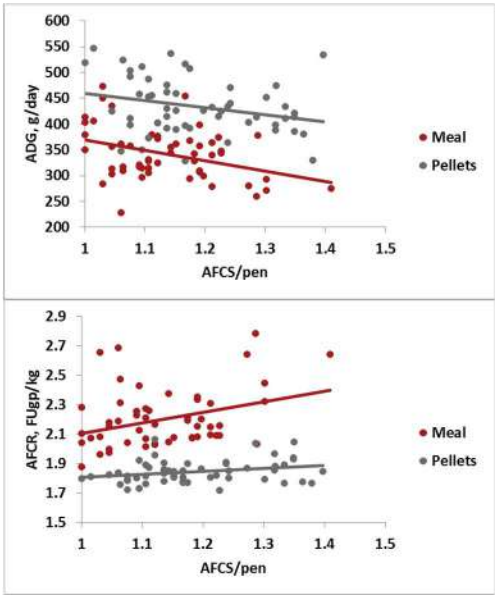

Figure 1. Pen wise daily gain and feed conversion ratio versus fecal consistency score in pigs fed meal or pellets

Discussion and Conclusion

This study was conducted in nursery pigs without severe diarrhea and with a low number of antibiotic treatments.
In spite of this, a more fluid fecal consistency at pen level was clearly associated with reduced daily gain and increased feed consumption per kg weight gain. The association was similar in pigs fed coarsely ground meal and finely ground pellets.

These findings support that fecal consistency is an important indicator of intestinal digestive function and gut health. This might be related to differences in microbiota affecting performance of individual pigs.

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Productive and economically effects of different crowding rates in pen and removal strategy on growth-to-finishing pigs

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Introduction

Individual pig productivity decreases as the overcrowding rate in pen increase. However, the fact that provide the necessary space to maximize growth is not necessarily associated with financial profitability. Therefore, the objective of this study was to determine the effects of different overcrowding rates considering the productive and economically significant parameters for the farm.

Materials and Methods

Experiment was conducted in a commercial finishing farm. The barn was naturally ventilated. Pens size were 15.3 m² and each pen was equipped with a stainless steel dry self-feeder and water was provided through a height-adjustable drinker. A total of 360 pigs were used. Pigs were penned by gender at arrival to the facility after nursery (0d). Twenty pens were allotted to initial floor space treatments of 1.02, 0.90 or 0.76 m^2, consistent with 15, 17 or 20 pigs per pen. Ten pens were allotted to initial floor space treatments of 1.02 and 0.90 m^2 (5 pens either). Meanwhile, 10 pens were allotted to initial floor space of 0.76 m^2. Of the 10 pens stocked at 20 pigs per pen, five pens had one pig removal strategy (topping). This strategy consisted to remove the 4 heaviest pigs at d 76. All pigs remaining in the experiment were marketed at d 83. Feed disappearance were determined on d 76 and 83 and individual weights were also collected on d 0, 76 and 83 to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed conversion (F/G).

Data were analyzed using the initial weight (d 0) as a covariable for all response variables. The least square means were used to report the significant relationship ($P < 0.05$) between weight at d 0 and variables. A second analysis was performed to test for the main effects with gender as the blocking factor. Individual treatment means were evaluated for differences using the Tukey adjustment comparisons.

Economic calculations were calculated accounting form premiums and discounts associated with varying individual BW, heavy (>120 kg), premium (90-120 kg), light (80-89 kg) and delayed (<80 kg). Individual revenue per pig was summed for the number of pigs in a pen to calculate the revenue per pen. Feed costs were used to calculate feed cost per pen and per pig based on the observed feed intake. Finally, to calculate the income over feed the total feed cost were subtracted from the total revenue.

Results

There was no sex by treatment interactions. Relationship between d 0 BW and weight at d 76 and 83, and average market weight per pen were significant ($P < 0.05$). The was no effect for within-pen BW variation (CV, %) and morbidity-mortality per treatment.

Table 1. Effects of crowding rate and topping strategy on productive and economically parameters.

			No topping	Topping
	1.02	0.90	0.76	
Avg body weight (BW) of pen prior to removals, kg				
d 0	37.98	37.93	36.59	36.21
d 76[3]	112.69	108.51	107.69	106.63
Avg body weight (BW) of pen after removal, kg				
d 76	112.69[a]	108.51[ab]	107.69[ab]	103.43[b]
d 83	117.28[a]	112.71[ab]	112.75[ab]	111.05[b]
Avg body weigth (BW) of marketed pen, kg[4]				
	117.28	112.71	112.75	112.68
Avg daily feed intake (ADFI) per pen, kg				
d 0 - 83	2.8[a]	2.52[b]	2.22[c]	2.24[c]
d 76 - 83	3.71[a]	3.20[b]	2.91[b]	3.47[ab]
Avg daily gain (ADG) per pen, kg				
d 0 - 83	0.941[b]	0.860[b]	0.874[ab]	0.875[ab]
d 76 - 83	0.675[b]	0.575[b]	0.747[b]	1.074[a]
Economic effect (Avg per pen)				
Total kg marketed	1688.20[a]	1756.96[a]	2073.94[b]	2069.26[b]
Income over feed, $	1865.22[a]	1975.75[a]	2500.62[b]	2605.59[b]

Conclusions and Discussion

Pigs with greater floor space (1.06 m^2) had improved ADG, ADFI and heavier average BW at market compared with other crowding rates. This productive performance agrees with previous research. From d 76 to 83, removing heavy-weight pen mates from a pen results in the remaining pigs having increased ADG an ADFI compared to pigs in intact pens. That suggests that relieving stocking pressure and providing additional floor space and resource access resulted in performance improvements.

Pens initially providing 0.76 m^2 floor space per pig have greater total kg marketed and revenue expressed on a pen basis. This was expected because other crowding rates had fewer pigs per pen. Data indicate that providing enough space for pigs to achieve their maximum growth was not the most economic.

It is a fact that the study confounded the effects of floor, feeder and water space. Therefore, it makes it harder to interpret the results and attribute the response to a single source. However, this experiment offers an answer to a real commercial farm situation in which productivity and economy are equally important.

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IMMUNOLOGY & VACCINOLOGY

Transcript quantification for cytokines in intestine from piglets vaccinated against *Escherichia coli* with Colidex-C, related to inflammatory infiltration and IgA-producing cells

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Introduction

The protection against *E. coli* has been classically based on sows and gilts vaccination. Only a few vaccines are registered to be used directly in piglets, using both, oral and parenteral administration routes. The objective of this work was quantifying cytokines transcript expression in intestine samples form piglets vaccinated against *E. coli* and *Clostridium* sp., and correlated them to presence of infiltrated cells and IgA-producing cells in tissue

Materials and Methods

One thousand eight hundred and ninety three piglets were vaccinated with COLIDEX-C (Vetia Animal Health, Spain) at 10th and 20th days of life. A control group (n=1869) remained unvaccinated. The animals were reared in the same nursery and finisher. At slaughter, samples from small intestine were freeze by immersion in 2-methylbutane refrigerated with liquid nitrogen. Moreover, samples were fixed in formalin for histopathology and cell infiltrate evaluation and quantification of IgA-producing cells by immunohistochemistry. The infiltrate was evaluated scoring the density of inflammatory cells in lamina propria from 0 (on infiltration) to 3 (severe infiltration). The IgA-producing cells were counting in 20 fields not overlapped. mRNA was isolated, cDNA synthetized and IFN-γ, IFN-α, TNF-α, TGF-β, IL-10, IL-12p35 y IL12-p40, were quantified using β-actin, cyclofilin and GAPDH as housekeepers for normalization. Relative quantification was done using the method described previously by Vandesompele et al[1], correcting the values to PCR efficiency.

Data were analyzed by means of Mann-Whitney's U test, and Spearman correlation. The difference in frequencies was assessed by Squared Chi test.

Results

There was higher infiltration score in vaccinated animals (p=0.028; Table 1), having and higher density of IgA cells in vaccinated animals compared with control; 25,78±0,84 and 10,38±0,41 (p<0.001), respectively. Small intestine from vaccinated pigs showed higher quantity of transcript for IFN-α (p<0.001), TNF-α (p=0.005), TGF-β (p=0.001) and IL12 p35 (p= 0.009) than those from unvaccinated piglets. No differences for IFN-γ, IL-10 and IL-12 p40 gene expression were found.

Interestingly, there was a correlation between TGF-β (r=0.5, p=0.01), IFN- α (r=0.39, p=0.011), TNF- α (r=0.384, p=0.011) and IL12-p35 (r=-0.315, p=0.039) expression and number of IgA-producing cells. There also was a significant different in the TGF- βquantitation comparing the animals with score 2 and 3 of inflammatory infiltration.

Table 1. Frequency of each infiltration score in intestine

	Infiltration score		
Group	1	2	3
Control	25^{a}	65^{a}	10^{a}
Vaccinated	5^{a}	55^{a}	40^{b}

Superscripts indicate statistically significant differences within main effect (p =0.028)

Conclusions and Discussion

The relation between cytokines expression revealed interesting features: IFN-α and TNF-α, hyperexpressed in vaccinated animals would recruit inflammatory cells which could explain the higher prevalence of infiltrate in vaccinated pigs. TGF-β stimulates the differentiation of B cells to IgA-producing cells[2] and the fact that higher inflamatory infiltration implies higher TGF-β gene expression suggest that the cells present are inducing chamge of isotype to IgA-producing cells in the plasmatic cells of the intestine. Even when vaccinated animals had higher number of cells in lamina propria of intestine, the productive performance was better (data not shown)

As conclusion, COLIDEX-C in piglets results in a different gene expression pattern for several cytokines, an ioncreased presence of inflamatory cells and a higher number of IgA-producing cells at slaughter.

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Effects of sow vaccination during lactation on fever and piglet performance

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Introduction

Sow vaccination could be associated with secondary adverse effects such as fever that may have a direct impact on sow feed intake, milk production and finally on piglet performance[1]. The vaccination plan for sows for the prevention of Erysipelas, Parvovirus and Leptospirosis, some of the main reproductive diseases, is during lactation. The objective of the present study was to compare the difference in fever events in sows associated with two reproductive vaccines against Erysipelas, Parvovirus and Leptospirosis on a commercial farm, as well as the effects on piglet performance after vaccination.

Materials and Methods

A total of 96 healthy multiparous sows and their litters was selected and randomly assigned to Control, Group 1 (G1) and Group 2 (G2) of 26, 33 and 23 sows, respectively. G1 was intramuscularly vaccinated with ERYSENG® PARVO/LEPTO (adjuvanted with HIPRAMUNE® G), whilst G2 was administered an alternative vaccine (adjuvanted with Amphigen®). Finally, the control group was inoculated with PBS. All administrations were performed 10 days after farrowing following the manufacturer's instructions.

Rectal temperature (RT) was recorded at vaccination and 3 times/day for 3 consecutive days. Fever events were considered when a RT value ≥ 40ºC was recorded in 2 or more registers. Piglets were weighed individually at vaccination day and 5 days post-vaccination (dpv).

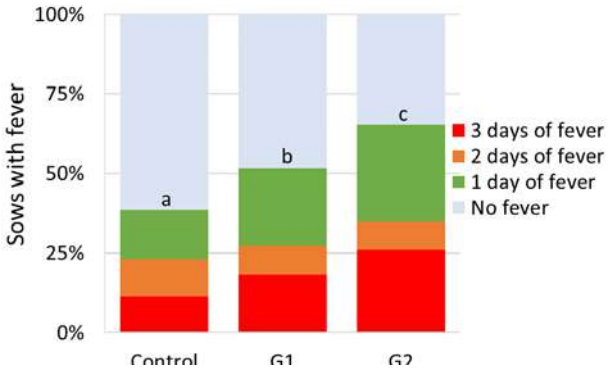

Figure 1. Percentage of animals classified according to the duration of fever per group (control, G1 and G2) during the study. Different letters show significant differences (ANOVA; *p-value* < 0.001).

Results

None of the animals had fever at the time of vaccination. However, after administration of the products, significant differences were observed between all groups both in the percentage of animals with fever (ANOVA; *p-value* < 0.001) and in the average duration of fever in these animals (Kruskal-Wallis; *p-value* < 0.001) (Figure 1). Thus, G2 had 1.3 days' duration of fever, which was significantly higher than the control group (0.7 days) and G1 (1.0 day) (Tukey test, *p-value* < 0.001).

Furthermore, the effect that this increase in temperature could have on piglet performance was evaluated. The results for average daily gain (ADG) showed statistically significant differences between the control group and G2 (Tukey test, *p-value* < 0.05), with a difference of 2.7 g/day between them (Figure 2). No significant difference was found between the control group and G1.

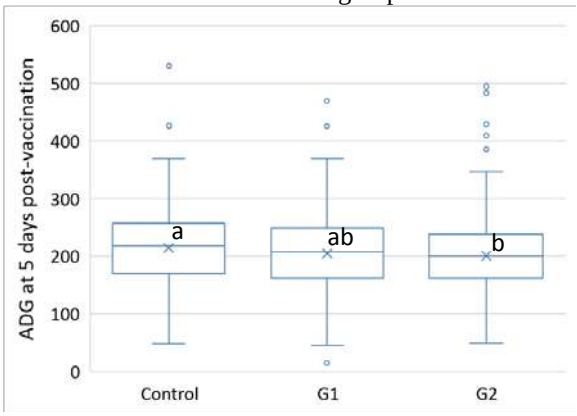

Figure 2. Piglets' average daily gain during the study period. Control, G1 and G2 had 290, 365 and 254 piglets, respectively. Different letters differ significantly (Tukey-test, *p-value* < 0.05).

Conclusions and Discussion

Although the vaccines compared in this study have been authorized for extensive use in lactating sows for the prevention of these reproductive diseases, not all of them show the same degree of fever events in sows and the consequences of this on piglet performance after vaccination, when injected under similar conditions compared with a control group during the lactation period. The data presented showed that G2 had the highest percentage of animals with fever and fever duration, reflecting a significant lower piglet performance at 5 dpv. In contrast, G1 showed a similar piglet performance to the control group, although a higher percentage of animals with fever and fever duration was observed. Therefore, these differences should be considered when defining a vaccine programme in lactating sows, due to the large number of vaccines administered in this period that can negatively affect the piglet performance of the litters.

Acknowledgments

HIPRASTAT team and S. Rodrigues-Chagas and L. Mendonça from Universidade Federal de Goiás.

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How to evaluate the inoculation point in piglets for Hipradermic®?

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Introduction

Needle-free vaccination through intradermal injection (NFID) is a less invasive technique and causes less anxiety and pain to the animal compared to other types of vaccination, amongst other benefits (elimination of broken needles, avoidance of iatrogenic spread).[1] However, this NFID can cause concern in users, regarding the correctness of the vaccine application, due to the difficulty in detecting the inoculation point in a visual inspection in some cases.

Intradermal injection involves the alteration of homeostasis at the inoculation point which would modify the normal thermal distribution of this area. Therefore, a method such as infrared thermography, which can visualize and measure the surface temperature[2] was thought to be able help to easily visualize the inoculation point when vaccinating with a NFID system such as Hipradermic®.[3]

The objective of the present study was to test and compare thermographic vs visual inspection as methods of evaluation of the inoculation point when vaccinating with the UNISTRAIN® PRRS vaccine in piglets using Hipradermic®.

Materials and Methods

A total of 70 healthy piglets of 4 weeks of age from a commercial PRRS-positive farm was selected. Sixty piglets were assigned to the V group and vaccinated intradermally with UNISTRAIN® PRRS using Hipradermic® (0.2 ml/dose). The other 10 piglets were the non-vaccinated (NV group), but the device had a similar physical contact with the animals to the V group. Visual inspection was performed by analyzing local reactions (inoculation point, papule, inflammation, redness, ulcer and/or scab) before vaccination, after vaccination, and 1h, 2h, 4h, 6h and 24h later. For the evaluation of the thermography, the FLIR ONE™ camera for Ios was used at the same times as visual inspection. All the data obtained were processed with FLIR® Tools software.

Results

Thermographic photos allowed the detection of a change in temperature in the anatomical area of the inoculation point in all the vaccinated piglets after vaccination. The change in temperature (difference between maximum and minimum temperature at the inoculation point; Dmax-min) for the V group after inoculation was 4.96±1.35, whilst the NV group did not show this variation (1.98±0.79). One hour after vaccination and later, these differences were not significant between groups (Figure 1).

Visual inspection allowed the detection of the inoculation point, papule or slight inflammation at different times (Figure 1). The highest percentage of vaccinated piglets with local reactions was detected at 2 hours post vaccination (83.93%) decreasing afterwards.

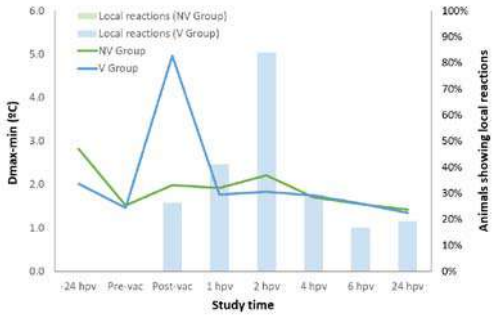

Figure 1. Evaluation of the inoculation point. Percentage of animals with local reaction (bars) and comparison of the difference between maximum and minimum temperature (Dmax-min) (lines) at the inoculation point in vaccinated and non-vaccinated animals at the different time points studied.

Conclusions and Discussion

This study compares the use of visual inspection and thermography as a method of detection of the intradermal inoculation in piglets under field conditions. The thermography allowed the visualization of the inoculation point after vaccination by ID as a "thermal footprint" (Figure 2).

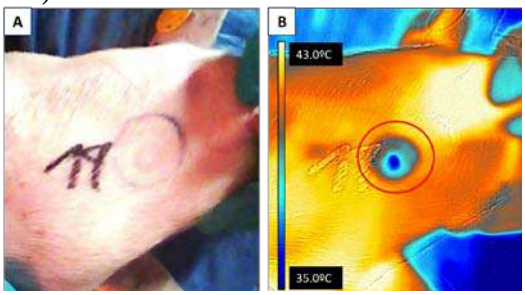

Figure 2. Picture at the time of vaccination. Real image of the piglet (A) and thermography (B) with the thermal footprint (inside the circle).

Thermography has specific new strengths in identifying correct inoculation in all piglets after vaccination (thermal footprint). However, visual inspection should be the chosen technique afterwards, especially at 2 hours post-vaccination. These techniques allow increasing user confidence by visualization of the correctness of the intradermal vaccination.

Acknowledgments

UCAM team

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Thermography as a new tool for the evaluation of Hipradermic® vaccination in sows in two different anatomical areas

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Introduction

Thermography is a non-contact, non-invasive technique that detects surface heat emitted as infrared radiation.[1] The colours of the images represent different temperatures, highlighting hot and cold spots and showing a map of the thermal distribution. Intradermal injection involves the alteration of homeostasis at the inoculation point[2] which would modify the normal thermal distribution of this area. It was therefore thought that infrared thermography could help to easily visualize the inoculation point when vaccinating with a needle-free intradermal injector such as Hipradermic®.[3]

The objective of the present study was to assess thermography as a method of evaluating the inoculation point when vaccinating with the UNISTRAIN® PRRS vaccine in sows using Hipradermic®.

Materials and Methods

A total of 130 healthy sows between 20-25 days' gestation from a PRRS-positive commercial farm was selected. Sixty sows were assigned to the V1 group and vaccinated intradermally in the neck area with UNISTRAIN® PRRS using Hipradermic® (0.2 ml/dose), whilst the other 60 animals were assigned to the V2 group, with vaccination performed in the perineum area. Finally, 10 sows were non-vaccinated (NV group), but the device had similar physical contact with the animal to the V group (5 sows were NV1 group and the other 5 were NV2 group in the neck and perineum areas, respectively).

Visual inspection was performed by analyzing the local reactions (inoculation point, papule, inflammation, ulcer and/or scab) previously to vaccination, post-vaccination, and 30 min, 4h, 6h and 24h later. For the evaluation of the thermography, the FLIR ONE™ camera for Ios was used at the same times as visual inspection. All the data obtained were processed with FLIR® Tools software.

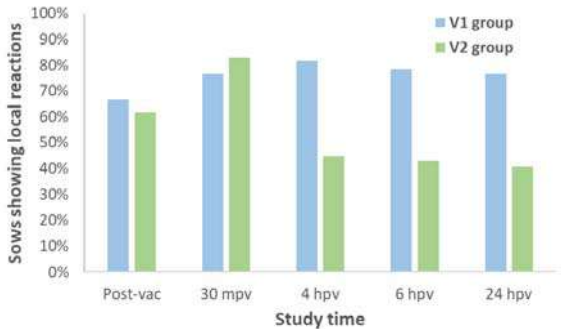

Figure 1. Percentage of animals with local reaction at the inoculation point in the vaccinated groups (V1 and V2) at the different time points studied.

Results

Visual inspection after vaccination allowed the detection of local reactions at the inoculation point in 66.7% and 61.7% sows from groups V1 and V2, respectively, although the highest percentage of vaccinated sows with local reactions was different depending on the anatomical area (Figure 1).

Thermographic photos detected the inoculation point (thermal footprint) in 100% of the vaccinated sows after vaccination, independently of anatomical area (Figure 2). The changes in temperature were based mainly on a reduction of the minimum temperature at the inoculation point for the V1 and V2 groups after inoculation (28.54±2.09 and 27.87±2.27, respectively), whilst the NV1 and NV2 groups did not show this variation (32.71±2.78 and 30.62±1.11, respectively). One hour after vaccination and subsequently, these changes were not significant between groups.

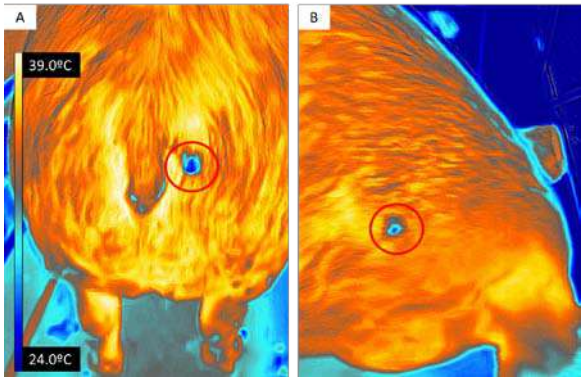

Figure 2. Thermal footprint (inside the circle) in vaccinated sows in the perineum (A) and neck (B) area.

Conclusions and Discussion

This study assesses the use of thermography as a method of detection of intradermal inoculation in sows under field conditions. A clear footprint at the inoculation point was detected in all vaccinated sows after the vaccination by ID (Figure 2). Therefore, thermography could be used as an easy alternative method of evaluation of the inoculation point when using the needle-free intradermal injector Hipradermic® in sows, with detection in 100% of vaccinated sows after vaccination.

Acknowledgments

UCAM team

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Effect of an anti-GnRF vaccine on growth performance of female finishing pigs in Thailand

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Introduction

Improvac® is an anti-Gonadotropin releasing factor (anti-GnRF) vaccine, which is well recognized for control boar taint and improve carcass quality in finishing male pigs (1). Meanwhile, anti-GnRF vaccine was also indicated for temporary estrus suppression of gilts (2) therefore improve performance problem that always found in the late finishing period particularly poor feed consumption, leads to decline of average daily gain (ADG) because the estrus cycling female pigs had less attention in feed consumption when compared to their opposite gender which leads leading to poor uniformity of final market weight in the same house (3, 4).

The objective of this study is to investigate growth performance including, average daily gain (ADG) and market weight of crossbred female finishing pigs which vaccinated with Improvac® compared to non-vaccinated standard gilts and physically castrated barrow under field conditions in Thailand.

Materials and Methods

Farm A: 321 crossbred finishing pigs (Large White x Landrace x Duroc) with average initial weight of 28.60 kg at 10 weeks old were randomly allocated by gender into 3 groups. Group 1 (T01), 132 female pigs were vaccinated with Improvac® (Subcutaneously, 2 ml per dose) for 1st dose at 11 weeks of age and 2nd dose at 17 weeks of age. Group 2 (T02) was 60 non-vaccinated female pigs and group 3 (T03) was 129 male piglets which were physically castrated at 3 days of age.

Farm B: 257 crossbred finishing pigs (Large White x Landrace x Duroc) with average initial weight of 23.15 kg at 9 weeks old were randomly allocated by gender into 3 groups. Group (T01), 73 female pigs were vaccinated with Improvac® (Subcutaneously, 2 ml per dose) for 1st dose at 12 weeks of age and 2nd dose at 17 weeks of age, while group 2 (T02) and group 3 (T03) were non-vaccinated female pigs and castrated male pigs respectively. There were 94 non-vaccinated female pigs and 90 male piglets which were physically castrated at 3 days of age.

All pigs in the treatment groups in these 2 farms were raised under the same conditions and received the similar diets. Average daily gain (ADG) was collected during the study period. All pigs in farm A and B were individually weighted on farm before sending to the slaughterhouse at 25 weeks of age for farm A, and 26 weeks of age in farm B.

Results and Discussion

The study outcome of average daily gain (ADG) and finishing weight are shown in Table 1 as below.

Table 1. ADG and market weight

Farm	Results	T01	T02	T03	p-value
A	ADG	797.53a (77.98)	755.24b (70.66)	827.93c (78.51)	<0.001
	Market weight	115.08a (8.89)	110.40b (7.59)	117.79c (9.13)	<0.001
	Day in barn	109a	110b	108a	<0.001
B	ADG	741.00a (69.50)	680.03b (93.37)	715.97a (92.81)	<0.001
	Market weight	111.22a (8.27)	104.22b (11.11)	108.20c (11.05)	<0.001
	Day in barn	119	119	119	-

*In the parentheses is the standard deviation.

Farm A: The Improvac® -vaccinated female pigs (T01) had significantly higher average daily gain and market weight than non-vaccinated standard gilts (T02) at 42.29 g/day and 4.68 kg respectively. Meanwhile, the physically castrated barrow (T03) had significantly higher average daily gain and market weight compare to group T01 and T02.

Farm B: The Improvac®-vaccinated female pigs (T01) had significantly higher average daily gain and market weight than non-vaccinated standard gilts (T02) at 60.97 g/day and 7 kg respectively and had significantly higher market weight than the physically castrated barrow (T03).

In conclusion, Improvac® can be an enabling tool to improve growth performance including average daily gain (ADG) and market weight by suppressing estrus of female finishing pigs in Thailand.

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Comparison of the safety and efficacy of two commercial modified live Porcine Reproductive and Respiratory Syndrome vaccines in late-term gestating sows

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Introduction

Introducing a new modified live Porcine Reproductive and Respiratory Syndrome (PRRSV) vaccine into sow herds endemic with PRRSV was indicated to be safe and could improve their reproductive performance in a published study (1). These improvements were also occurred under heterologous challenge conditions (2). Therefore, the aim of this study was to investigate the safety and reproductive performance in sow herds by introducing a new modified live PRRSV vaccine, Fostera™ PRRS, to sows during their late term of gestation which were on another modified live PRRSV vaccine with mixed results at the time of study.

Materials and Methods

128 sows were randomly divided into two groups. In group 1 (T01), 62 sows were vaccinated with Fostera™ PRRS (P129 strain, Zoetis) intramuscularly, 2 ml per dose at 5 weeks before farrowing while in group 2 (T02), 66 sows were vaccinated with Ingelvac PRRS® MLV (VR2332 strain, Boehringer Ingelheim) intramuscularly, also 2 ml per dose at 5 weeks before farrowing. The reproductive performance parameters such as total born, born alive, stillborn, mummified, litter birth weight, birth weight, pig wean per litter, pre-weaning mortality, litter weaning weight, weaning weight and average daily litter weight gain (ADLWG) were all recorded. Meanwhile, serum neutralization assay (SNT) and Real-Time Polymerase Chain Reaction (rt-PCR) were performed on the sera samples. Colostrum samples of ten sows in each group were randomly collected to determine for PRRS antibody titers by SNT. The viremia status would be evaluated by rt-PCR from piglets' sera at 1 day of age, which were collected from tail docking process.

Results and Discussion

Clinical Observation: No local or systemic adverse reaction related to the post-vaccination were observed in both groups throughout the study. From this result, the administration of both modified live PRRSV vaccines to sows at 5 weeks before farrowing was considered safe.
Serum Neutralization: The mean of SN titer of Fostera™ PRRS (T01) group and Ingelvac PRRS® MLV (T02) group is shown as Table 1.
The percentage of pre-weaning mortality in group T01 was significantly lower than group T02. Meanwhile, the piglets in group T01 had significantly heavier litter weaning weight than group T02. However, other parameters in this study had no significant difference between two groups.

Table 1. Mean of SN titer of Fostera™ PRRS group (T01) and Ingelvac PRRS® MLV group (T02). The SN titer in colostrum of sows in FosteraTM PRRS group (T01) was significantly higher than Ingelvac PRRS® MLV group (T02).

Immunological parameter	Group		p-value
	T01	T02	
SN titer (log 2)	6.69 (3.24) [a]	1.50 (2.47) [b]	<0.001

*In the parentheses is the standard deviation.

Viremia status: The PRRSV antigen was not found in the piglets' serum in both groups. The reproductive performance is shown in Table 2. Reproductive performance of Fostera™ PRRS group (T01) and Ingelvac PRRS® MLV group (T02).

Parameter	Group		p-value
	T01	T02	
Parity	4.57	4.16	0.36
Total born/litter	14.38	14.27	0.85
Born alive/litter	12.68	12.48	0.67
% Stillborn/litter	8.95	11.74	0.12
% Mummy/litter	2.06	0.9	0.11
Litter birth weight (kg)	18.67	18.12	0.48
Birth weight (kg)	1.5	1.51	0.87
Pig weaned/litter	11.19	10.79	0.13
% Pre-weaning mortality	9.47[a]	15.93[b]	0.03
Litter weaning weight (kg)	79.58[a]	71.95[b]	0.01
Weaning weight (kg)	7.09	6.68	0.07
ADLWG (g/day)	1,977.36	1,909.23	0.45

In conclusion, Fostera™ PRRS, was safe for use in the late term of gestating sows. Moreover, the benefit of high SN titer level in colostrum of the sows in Fostera™ PRRS group (T01) was increasing the opportunity of the piglets to receive the maternally derived antibody, which was direct benefit to the piglet's passive immunity against PRRSV in the early life (3) and indirect benefit in piglets' health and performance improvement.

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A comparison of the field efficacy of a single-dose Fostera™ PCV MH versus a two-dose PCV2-*Mycoplasma hyopneumoniae* vaccination regimen in Thailand

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Introduction

Swine producers in Thailand are routinely administering a two-dose vaccination regimen which consists of an initial single-dose bivalent PCV2 - *Mycoplasma hyopneumoniae* vaccine followed by a booster dose of monovalent PCV2 vaccine to decrease the risk of PCV2 infection progressing to PCVAD and respiratory complication in finishing pigs. Fostera™ PCV MH (FPCVMH) has been successfully adopted in several farms in Thailand to reduce PCV2 viremia and Mycoplasmal nasal shedding, lower the lung and lymphoid lesion scores under the field conditions as scientifically proven in other markets (1,2). This study aimed at comparing the serological response, viremia and nasal shedding reduction, and the overall growth performance in pigs vaccinated with either a single-dose of FPCVMH or the existing two-dose vaccination program implemented in a commercial pig farm.

Materials and Methods

A total of 29 pigs from a commercial farm were randomly divided into 2 groups (Table 1). At 4 and 7 weeks of age, all pigs in Group 1 (n=14) were vaccinated intramuscularly with 2.0 mL per dose of a bivalent PCV2-*M. hyopneumoniae* vaccine (Vaccine A) followed by a monovalent PCV2 Vaccine (Vaccine B) with 0.5 mL per dose which is a routine two-dose vaccination program. Pigs in Group 2 (n=15) were vaccinated with 2 mL per dose of FPCVMH at 4 weeks of age following the label instruction for a single-dose regimen.

Table 1. Experimental design.

Group	N	Vaccine	Timing of vaccination (weeks of age)	
			4	7
1: a two-dose vac. regimen	14	1st dose (Vac A) 2nd dose (Vac B)	2 ml	0.5 ml
2: a single-dose FPCVMH	15	Fostera™ PCV MH	2 ml	-

All pigs were raised under same conditions. Blood and nasal swab samples were collected at 3, 9, 15 and 22 weeks of age. Presence of anti-PCV2-IgG antibody were evaluated by Synbiotics SERELISA® PCV2 Ab Mono Blocking ELISA. PCV2 DNA copy numbers in blood was quantified by real-time PCR at 15 weeks of age where serum samples were sequentially pooled 3 per 1 PCR sample. For the detection of *M. hyopneumoniae*, the nasal swab samples were tested by nested PCR. Average daily weight gain (ADWG) and number of dead or culled pigs were also recorded (Table 2).

Results and Discussion

Anti-PCV2-IgG antibody levels

At the time of the first vaccination, pigs in both groups were PCV2 seropositive. Anti-PCV2-IgG antibodies in Group 2 seemed higher than control group (two-doses). As expected, both two-doses and single-dose groups showed similar amount of anti-PCV2-IgG antibodies in each week of age with the change in the same direction. (Figure 1.).

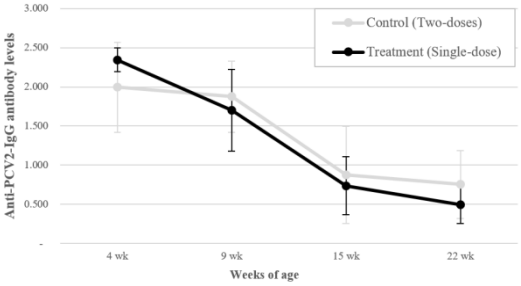

Figure 1. Group mean S/P ratios and standard deviation for anti-PCV2-IgG antibodies responses at 4, 9, 15 and 22 weeks of age.

Table 2. Comparison of Group 1 and 2 for the mean amounts of PCV2 genomic copies, prevalence of *M. hyopneumoniae* nasal shedding and ADWG.

	Weeks of Age	Group 1 (Two-doses)	Group 2 (Single-dose)
No. pigs of PCV2 viremia	15	5/5	3/5
PCV2 genomic copies		2.3 ± 0.4	2.6 ± 0.5
No. pig of *Mycoplasma hyopneumoniae* nasal shedding	3	0/5	0/5
	9	5/5	5/5
	15	5/5	5/5
	21	4/5	5/5
ADWG	4 - 23	618 ± 31	595 ± 45

No significant difference (p<0.05) between Group 1 (Two-dose regimen) and Group 2 (a single dose of Fostera™ PCV MH)

In conclusion, the present study demonstrated that a single-dose vaccination with Fostera™ PCV MH has comparable efficacy to the existing two-dose PCV2 vaccination regimen with Vaccine A and Vaccine B. Therefore, Fostera™ PCV MH can be a substitute vaccine for swine producers in Thailand to protect their pigs from the two critical pathogens PCV2 and *M. hyopneumoniae*, with the convenience of a safe, single injection, without sacrificing standard efficacy and economic benefits in terms of growth performance.

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Virulence and antibody responses of U.S. PEDV strains in pigs of different ages

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Introduction

Porcine epidemic diarrhea virus (PEDV) is the causative agent of porcine epidemic diarrhea, an enteric disease affecting global swine industry.[1] Two main strains of PEDV circulate in U.S. swine herds (non S-INDEL and S-INDEL PEDVs).[2] While studies have demonstrated that S-INDEL PEDV is less virulent than non S-INDEL PEDV in neonatal conventional pigs[3]; little is known about PEDV virulence in older pigs. This study aimed to compare the virulence and antibody responses of these two PEDV strains in post-weaning pigs of three different ages.

Materials and Methods

Thirty 3-week-old ("weaned"), thirty 8-week-old ("grower"), and thirty 23-week-old ("finisher") PEDV naïve pigs were included in this study. Pigs within each group of age were subdivided into 3 subgroups (10 pigs/group) and orogastrically inoculated with PEDV isolate USA/IN19338/2013 (non S-INDEL), USA/IL20697/2014 (S-INDEL), or sham inoculated with culture medium. Five (5/10) pigs within each subgroup were randomly selected for necropsy at 4 DPI and the remaining pigs were necropsied at 28 DPI. Viral load from fecal swabs, oral fluids, and tissues were determined by PEDV N gene-based qRT-PCR. Serum neutralizing antibody (NA) was measured by FFN assay using non S-INDEL PEDV as the indicator virus. Serum IgG and oral fluid IgA antibodies were measured by PEDV S1 protein-based FMIA

Results

The mean PEDV genomic copies from fecal swabs are shown in Figure 1. In "weaned" pigs, non S-INDEL PEDV had longer duration of fecal shedding and significantly higher fecal and oral fluid virus loads than S-INDEL PEDV. In "grower" pigs, S-INDEL PEDV had longer fecal shedding than non S-INDEL PEDV; S-INDEL fecal virus load was significantly higher than non S-INDEL PEDV at 7 and 14 DPI but was opposite at 10 DPI. In "finisher" pigs, the onset and viral load of non S-INDEL PEDV fecal shedding was earlier and higher than S-INDEL PEDV. For non S-INDEL PEDV, the onset and viral load of fecal virus shedding was earliest and highest in "weaned", followed by "grower" and "finisher" pigs. S-INDEL PEDV trended similarly across age groups. Serum NA and IgG responses, and oral fluid IgA responses induced by non S-INDEL PEDV were greater than S-INDEL PEDV in both "weaned" and "finisher" pigs, while the difference of antibody responses induced by two PEDV strains in "grower" pigs was variable and dependent on the immunoassay platform used for testing. Non S-INDEL PEDV induced similar NA responses in the three groups of age, stronger serum IgG response in "weaned" pigs than in "grower" and "finisher" pigs, and stronger oral fluid IgA response in "finisher" pigs than in "grower" and "weaned" pigs. Interestingly, S-INDEL PEDV induced a stronger antibody response in "grower" pigs than in "weaned" and "finisher" pigs.

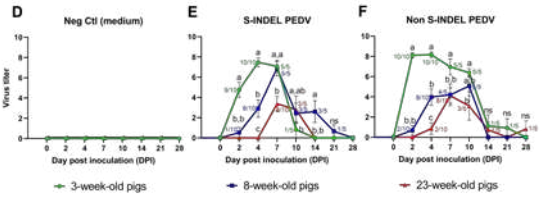

Figure 1. Virus shedding[1] in rectal swabs of pigs at different ages inoculated with different U.S. PEDV strains. [1]Determined by PEDV qRT-PCR. Titers (log_{10} genomic copies/ml) at each time point were the mean of all available pigs in each group. Data labels (fractions) represent the number of PEDV PCR positive pigs detected. Standard error bars are shown and significantly ($P \leq 0.05$) different values for each DPI are indicated (^{a,} ^{b,}).

Conclusions and Discussion

Results suggested that PEDV virulence is pig age-dependent (more severe in younger pigs) and virus strain-dependent. Non S-INDEL PEDV was more virulent than S-INDEL PEDV in "weaned" and "finisher" pigs, but the differences on virulence between PEDV strains were less distinct in "grower" pigs. Antibody response of PEDV is also both virus strain-dependent and pig age-dependent. The data from this study provide some guidance on selecting appropriate PEDV strain to induce antibody response in different ages of pigs and on selecting PEDV strains for vaccine development.

Acknowledgments

Funding provided by Iowa Pork Producers Association

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Why is almost any pig "latently" infected with PCV2 in spite of vaccination against the virus since 2008?

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Introduction

In 2015 (Klausmann et al., 2015), for the first time, we put forth the theory that the major pathogenicity mechanism of porcine circovirus type 2 (PCV2) is "immune tolerance". Testing this theory further supported this view, in as much as vaccination barely affected infection prevalence and about 100% of investigated embryos were "latently" infected with low viral amounts, as were pigs prior to the age of immune competence (Sydler et al. 2016). This high prevalence raised the question whether gametes directly carry PCV2 into the developing pig embryo. A single virus of PCV2 might be sufficient for infection success, therefore we needed a) a read out system capable of detection and localization of virus accumulation b) anatomically distinguishable pigs' sexual organs for visualization and c) independence of perturbation by external PCV2 infections (e.g. from sexual activities). These criteria fit best with the age of stillborn pigs, and due to their ready availability this also fit well with the 3R concept. Therefore, we analyzed female and male stillborn pigs with immunohistochemically PCV2-negative genital tissues, from farms with no PCV2-AD symptoms, by fluorescence *in situ* hybridization (FISH).

Materials and Methods

We present our findings from 55 stillborn fetuses (31 m, 24 f). These pig fetuses were from 6 farms without porcine circovirus diseases or any PCV2 associated diseases: namely from farm A, 15 m and 15 f; farm B, 3 m and 2 f; farm C, 2 m and 2 f; farm D, 3 m and 1 f; farm E, 4 m and 2 f and farm F, 4 m and 1 f. Their genital organs, spleens and thymi were investigated by necropsy, immunohistochemical analysis and FISH (Khaiseb et al., 2011) for PCV2. The bulk of FISH sections was analyzed with the Hamamatsu's NanoZoomer 2.0 HT with fluorescence option. To confirm and refine the analysis we also used a Leica fluorescence microscope and confocal microscopy. (Additionally, we analyzed these 54 stillborn fetuses genital tracts, as well ovulated eggs from 3 hysterectomized sows, by PCR (presented in our sister poster).

Results

PCV2-positive FISH signals were found regularly in piglets from all farms. Signals were much higher in male genital tissues than female genital tissues. Please see the figure in our poster, which depicts in
color, a "high"-level latently infected testis, which shows fluorescent hybridization signals of the PCV2 specific oligonucleotide and the oligonucleotide for double-stranded PCV2 DNA, and the overlay of these signals, illustrating signal co-localization.
While we found hardly any visible PCV2 infections by FISH in female sexual organs, their sexual organ-associated lymph nodes were regularly saturated with PCV2 infected cells, visible only by FISH (when lymphatic tissue was also available on the slides).

Conclusions and Discussion

We could find no evidence that the gametes themselves were directly PCV2 infected either in male or in stillborn female pigs. Nevertheless, genital tract associated lymphatic tissues, mainly in females and in male interstitial cells of the testicles and epithelial cells of accessory sexual glands, tested positive for PCV2 infected cells by FISH.
This anatomically close association of PCV2 infection with germ line cells leaves open the possibility that gametes carry PCV2 to the location of fertilization in the oviduct, via a kind of a piggyback mechanism or in associated fluids. Actually, we have some data that would support either one of the models above (see please our sister poster).

Acknowledgments

This work was financially supported by the ***Boehringer Ingelheim*** award. We thank the DT Druck-Team, Wetzikon, CH, for professional graphic support.

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At what stage of the pig embryonic development do PCV2 infections occur relevant to its "immune tolerance" pathogenicity?

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Introduction

Infection of porcine circovirus type 2 occurs before immune competence (Sydler et al., 2016) during pig fetal ontogeny as known from the classical sense of the term "immune tolerance" (Oldstone et al., 2009). This is also what we proposed to be the driving force for PCV2 pathogenicity (Klausmann et al., 2015). We found also in the manuscript, Sydler et al. 2016, four embryos younger than 55 days. They were infected with PCV2 prior to immune-competence. These findings let us to question at what early developmental stage pig embryos are infected with PCV2? In the sister poster at this IPVS-meeting, we show PCV2 infected cells close association with pig's gametes. Even though Kim et al., 2003 and Bielanski et al., 2004 found in close association with gametes PCV2, most research scientists still think that PCV2 infections of the unborn fetus occur through the placenta if at all. We present here new evidence for the alternative interpretation.

Materials and Methods

Three sows were chosen for embryos collections, as they would have been anyway slaughtered due to their age. These three sows were artificially inseminated twice during estrus after weaning of their piglets. Sows were slaughtered and their uterus was removed 3 days after 2nd inseminations. The uterine horns were ligated at the tip of the horn about 8 cm distal from the oviduct junction. In the middle of the oviduct a small hole was pricked. Through this hole, the fertilized embryos were collected by rinsing into the ligated horn tips and finally collected into 4-well Petri dishes where the embryos were 3 times washed to remove loosely attached PCV2. The embryos as well as the primary rinse and the 54 stillborn fetuses (31 male (m), 23 female (f) stillborn fetuses presented on our IPVS-sister poster) were investigated by nested PCR for the presence of PCV2 DNA. For first amplification the oligonucleotide pair PCVfo2f2 5' GGT GCT GCC GAG GTG CTG 3' and PCVfo2r2 5' GGA GGA TTA CTT CCT TGG TAT TTT GG 3' were used. For second amplification we used oligonucleotide pair describe in Wiederkehr et al., 2009. The PCR amplification was performed with the help of PhusionFlash (ThermoFisher) polymerase, which has proofreading activities. Each step of the nested PCR was first optimized on cloned whole PCV2 sequences (Klausmann et al., 2015).

Results

Two days after insemination of the three sows we received seven multicellular clumps that we call for simplification embryos. Six of these embryos were PCR positive for PCV2 sequences. The primary fluid of the rinse was also tested for the presence of PCV2. One out of these three rinses contained PCV2 sequence.
We analyzed also the genital tract organs of the 54 stillborn fetuses for the presence of PCV2 by nested PCR (This is the same group of stillborn fetuses that we had already analyzed on the sister IPVS-poster by FISH). We found nine out of 23 female stillborn fetuses, which carried PCV2 by PCR. In the 31 male stillborn fetuses we could detect in six fetuses PCV2 DNA.
Even though the amplified sequence fragment between the oligonucleotides contains only 137 nucleotides of the virus capsid, it clearly allows us to distinguish PCV2a from PCV2b and PCV2d group members. Thus, we found all sequences detected by PCR belong to PCV2b group members.

Conclusions and Discussion

We think that the primary infection with PCV2 occurs in the fertilization process as the gametes bring associated virus into the early development of a new pig embryo. This would predate placenta formation.
It is also interesting to note that we have found a PCV2d group member as early as 1999, however, they could not prevail so far against the PCV2b group members in Switzerland.

Acknowledgments

This work was financially supported by the ***Boehringer Ingelheim*** PCV2 award.

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Effect of a one-shot combination versus a monovalent vaccine on PCV2 viremia and performance parameters in a German pig farm

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Introduction

Vaccination against Porcine Circovirus type 2 (PCV2) has been very successfully worldwide (1) due to very effective disease control, reduction of PCV2 viral load and reduction of the number of viremic animals (2,3,4). Despite the general very positive effects, commercial PCV2 vaccines differ in several features, including
the use of different adjuvants which may result in a remarkable effect on pig safety and performance.

Materials and Methods

In a German breeding farm (pig production) all suckling pigs were routinely vaccinated only against *Mycoplasma hyopneumoniae* (M. hyo) with a 2-dose vaccine at 3 and 21 days of age. No PCV2 vaccine was used before. The study was performed in two time periods including two vaccination groups each (table 1). In the first phase piglets in the control group A (n=263) were vaccinated with the routine M. hyo vaccine only, and the piglets in the experimental group B (n=261) received Suvaxyn® M. hyo (Zoetis) at 1 week of age, Suvaxyn® Circo+MH RTU (Zoetis) at 4 weeks of age. In phase 2 piglets in the positive control group C (n=259) received an alternative PCV2 vaccine at 21 days of age in addition to the M. hyo vaccination routine program, and pigs in the experimental group D (n=262) received the same vaccination program as group B (table 1). Serum samples were collected from pigs at weaning, 6 (mid nursery), 10 (end of nursery) and 22 weeks of age. Samples were tested for PCV2 with a real-time PCR test. All pigs were weighed at enrollment, 4 weeks later, at the end of nursery and at the end of fattening. Lung slaughter checks were performed at the abattoir.

Results

No differences in lung lesions between treatment groups were found at slaughter. PCV2 detection rates in blood samples (table 2) resulted in significant differences between groups A and B from 6th week of age onwards (p=0.032, p=0.000, p=0.000). In phase 2, the total number of viremic animals (n=22) was lower than the number of positive animals (n=105) in phase 1. There was a significant difference in the number of viremic animals between the vaccination groups at the end of nursery in phase 2 (p=0.018). The average daily weight gain (ADWG) (table 3) of group B (combination M. hyo+ PCV2 vaccine) was significantly better (+16 g, p=0.38) than group A (only vaccinated against M. hyo). This resulted in additional final average weight of 3.1 kg at 22 weeks of age. Within phase 2 pigs in group D had a significantly better ADWG than the vaccinated with the alternative PCV2 monovalent vaccine (group C) (p=0.01, table 3).

Table 1. Vaccination schemes.

Age (d)	Experimental groups A	B	C	D
3	M. hyo vaccine	Suvaxyn M. hyo	M. hyo vaccine	Suvaxyn M. hyo
21	M. hyo vaccine	Suvaxyn Circo+ MH RTU	M. hyo + PCV 2 vaccine	Suvaxyn Circo+ MH RTU

Table 2. Serum PCV2 PCR results.

Positives (%)	Experimental groups A	B	C	D	P-value
Weaning	8.9	4.4	6.7	6.7	
Week 6	2.2^{A}	9.1^{B}	6.8	13	AB 0.032
Week 10	88.2^{A}	30.8^{B}	0^{C}	12.2^{D}	AB 0.000 CD 0.018
Week 22	90.6^{A}	30.8^{B}	0	7.7	AB 0.000

Table 3. Average daily weight gain of the groups.

ADWG (g)	Experimental groups A	B	C	D	P-value
Minimum	228	348	326	301	
Mean	583^{A}	599^{B}	545^{C}	563^{D}	AB 0.38 CD 0.01
Maximum	779	796	745	840	

Conclusions and Discussion

It can be assumed, that due to the fact that only 50% of the pigs were vaccinated against PCV2 in phase 1 and all in phase 2, there were less PCV2 viremic pigs in the second phase. Interestingly Group C had no viremic animals in weeks 10 and 22, but resulted in the lowest ADWG. A possible explanation could be an increased inflammatory post-vaccination response to the alternative vaccine in Group C, compared to other treatment groups. This stress factor could have reduced feed intake and therefore negatively impacted the growth of pigs in that treatment group. This summary only covers production and laboratory results, additional data will be included in a full detailed publication.

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Evaluating a novel immunostimulant for improvement of homologous hemagglutinin inhibition (HI) titers in gilts vaccinated with a quadrivalent killed whole virus Influenza A Swine (IAV-S) vaccine

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Introduction

Vaccination is a critical component of health management plans for swine of all ages and production purposes. Pre-breeding vaccination of gilts is a common method of developing acquired immunity prior to breeding, pregnancy and parturition. Betaglucans are naturally occurring polysaccharides with demonstrated abilities to increase host immune defense by activating complement system, enhancing macrophages and natural killer cell function.[1] Increasing attention is being given to the ability of beta-glucan to stimulate the immune system of commercially farmed swine, but substantial additional research is needed to optimize this practice.[2]

Materials and Methods

60 x 6 month old purebred large white gilts were housed in a commercial gilt development unit and administered a commercial diet with contained either the labeled rate of a commercially available betaglucan product (Treatment) or no betaglucan (Control). Following 30 days of feeding both Treatment and Control groups, all gilts were vaccinated with a quadrivalent killed whole virus IAV-S vaccine (Cambridge Laboratories, Inc., Worthington, MN) and serum was collected from all animals via jugular venipuncture. 30 days post- vaccination, serum was again collected from all gilts. Serum IAV-S titer was evaluated for all samples via homologous HI (University of Minnesota Veterinary Diagnostic Lab, St. Paul, MN). The effect of Treatment on homologous HI titers were analyzed using a mixed effect two-way ANOVA model followed by Tukey-Kramer adjusted t-test (SAS® Enterprise Guide 4.3, Cary, NC, USA).

Results

Serum titers in the Treatment group were significantly improved for a single isolate and numerically improved for all isolates.

Table 1. Titer change: Post minus Pre-Vaccination

HI Strain	Control	Treatment	p-value
IAV-S Isolate 1 (H1)	140	171	0.43‡
IAV-S Isolate 2 (H1)	71.6	164	0.03‡
IAV-S Isolate 3 (H1)	212.8	254	0.47†
IAV-S Isolate 4 (H3)	208	250	0.40†

†p-value from non-parametric test.
‡p-value from ANOVA.

Conclusions and Discussion

Feeding betaglucans during the time of IAV-S killed whole virus vaccination demonstrated the ability to significantly increase titer response. While the 3 other isolate titers evaluated were only numerically improved, the consistency of the results warrants further research into the ability of betaglucans to improve vaccination quality in commercial swine. Feeding betaglucans during vaccination is a promising strategy to improve vaccination impact.

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Comparative study of safety and immune response of two commercial *Actinobacillus pleuropneumoniae* vaccines

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Introduction

The objective of this trial was to compare the safety profile and the immune response induced by two commercial vaccines against *Actinobacillus pleuropneumoniae.*

Materials and Methods

Forty-five piglets of 10 weeks of age were divided in three study groups:

- Group A: vaccinated with Coglapix® (Ceva Salud Animal) at 10 weeks of age and revaccinated 4 weeks later (n=15)
- Group B: vaccinated with Porcilis® App (MSD Animal Health) at 10 weeks of age and revaccinated 4 weeks later (n=15)
- Group C: control not vaccinated (n=15)

All animals were bleeded at 10, 14 and 18 weeks of age. Samples were processed at the R&D Service Lab (MSD Animal Health, Boxmeer) for the following parameters:

- ApxI, ApxII, Apx III and OMP: in home titration test, that expresses results in log2 titer values
- ApxIV: Idexx Apx IV ELISA test

Rectal temperature was recorded at vaccination (t0) and 24h later (t24), as well as local (observation and palpation) and systemic reactions (observation).
All data were statistically analyzed (two-factor ANOVA test).

Results

No local nor systemic reactions were observed in any animal of any group.
It was not observed a significant increase in rectal temperature of any of the vaccinated groups, compared to the control one, although 24h postvaccination rectal temperature was statistically higher in all three study groups than at time of vaccination (Table 1).

Table 1. Rectal Temperatures at t0 and t24 (ºC ± SD)

	T0	T24
Group A	39,46±0,49	39,78±0,41
Group B	39,59±0,41	39,65±0,40
Group C	39,44±0,38	39,99±0,40

Immune response data are shown in Table 2.
Statistical differences were observed in titration of ApxI, ApxII, Apx III and OMP between Porcilis® App and Control at 14 and 18 weeks of age. At 18 weeks, statistical differences between Coglapix® and Control were only found in ApxI, ApxII and OMP. Although numerical values were higher for Porcilis ®App group, statistical differences between Porcilis® App and Coglapix® were only found in ApxI titres at 18weeks of age.

Table 2. ApxI, ApxII, Apx III and OMP immune response

		10w	14w	18w
ApxI	A	6,81±0,33	7,83±0,92^{a}	10,01±1,09^{a}
	B	6,95±0,45	9,45±1,73^{b}	11,97±1,61^{b}
	C	7,37±1,01	6,96±0,45^{a}	7,45±0,46^{c}
ApxII	A	8,9±0,85	9,50±1,26^{ab}	10,36±1,20^{ab}
	B	8,26±0,65	10,09±0,83^{a}	12,35±10,07^{a}
	C	8,71±0,80	8,88±1,08^{b}	9,88±1,19^{b}
ApxIII	A	9,44±1,34^{a}	10,32±1,22^{a}	10,51±0,72^{a}
	B	9,03±0,96^{a}	10,06±0,66$_{a}$	10,80±0,61^{a}
	C	7,58±0,91^{b}	7,72±1,35^{b}	7,65±0,68^{b}
OMP	A	7,76±0,74	10,23±1,05^{a}	9,61±1,02^{a}
	B	7,92±0,87	9,95±0,90^{a}	10,14±1,14^{a}
	C	8,00±0,79	7,92±0,94^{b}	7,71±0,75^{b}

a,b,c Different superscripts in same column indicate statistical differences (p<0.05)

Apx IV results showed a certain level of MDA (at 10w, 5 positives in group A, 4 positives in group B and 6 in group C), although at 18 weeks only one animal of Group A was positive to ApxIV.

Conclusions and Discussion

As previous experiences have shown (1), the results show that some differences in immune response can be detected between different commercial vaccines against *A. pleuropneumoniae*. The data obtained in this study indicate that Porcilis® App is a safe vaccine that induces a strong immune response post-vaccination. The differences observed, especially in antibodies against ApxI, might be of importance, especially when challenge strains are from serotypes 1,5,9 10 and 11

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Field study to evaluate safety and seroconversion of a new intramuscular vaccine against *Lawsonia intracellularis*

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Introduction

Lawsonia intracellularis (L.i.) is a worldwide distributed pathogen affecting swine population. A recent European study revealed that more than 90% of the farms reporting enteric disorders during growing period, showed presence of the bacteria either in faeces or serum (antibodies) (1). Hence, tools against this pathogen are needed. The aim of this study was to evaluate safety characteristics and seroconversion, provided by a new intramuscular vaccine, available in the EU market.

Materials and Methods

The study was carried out in 2 commercial farms producing piglets until 10 weeks of age. In both production units the protocol developed was the same. A group of piglets between 3 and 4 weeks of age were selected previous weaning (Farm 1: 30 piglets; Farm 2: 60 piglets), and all of them were individually identified, blood sampled, and their rectal temperatures were recorded (ºC). Then, different treatment categories were designed in order to have a control group (C: not vaccinated), as well as a group vaccinated with Porcilis® Lawsonia (MSD A.H.) alone (PL), and the combination of Porcilis® Lawsonia reconstituted in Porcilis® PCV Mhyo (MSD A.H.) (PLP) (Table 1: animals/group).

Table 1.

	Control	Porcilis® Lawsonia	P. Lawsonia + PCV Mhyo
FARM 1	15	15	0
FARM 2	20	20	20

Rectal temperatures were then recorded individually at 6 and 24 hours postvaccination, to assess the safety of the *L.i.* vaccination. Again, all piglets were bleeded at vaccination and 4 weeks postvaccination (Farm 1); and at vaccination and 3 and 6 weeks postvaccination (Farm 2). Serum samples were sent to the laboratory and stored until analysis under the same conditions. The diagnostic kit SVANOVIR® L. intracellularis/Ileitis-Ab (Boehringer Ingelheim Svanova) was used. Two-way mixed ANOVA (95% CI) were done to analyze the data for both farms regarding temperature records.

Results and Discussion

Comparison of rectal temperatures and seroconversion are shown in Tables 2 and 3 respectively.

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Table 2.

	Rectal temperature (ºC)		
Timing	Groups	Farm 1	Farm 2
0h	C	39.7[a]	40.1[e]
	PL	39.5[a]	39.5[f]
	PLP		39.7[f]
	p-value	*p=0,172*	*p<0,000*
+6h	C	39.4[b]	39.7[g]
	PL	40.2[c]	40.3[h]
	PLP		40.3[h]
	p-value	*p<0,001*	*p=0,006*
+24h	C	39.6[d]	39.3[i]
	PL	39.5[d]	39.1[i]
	PLP		39.3[i]
	p-value	*p=0,311*	*p=0,094*

Different letters in same farm/timing indicates statistical differences

An increase in average rectal temperature appears 6 hours postvaccination in all vaccinated groups (+0.8ºC Farm 1; +0.6ºC Farm 2). These statistical differences disappear 24 hours postvaccination.

Table 3	Seroconversion (% + samples)		
Weeks age	Groups	Farm 1	Farm 2
3 w.	C	0%	0%
	PL	0%	5%
	PLP	-	0%
6 w. Farm 2	C	8%	0%
7 w. Farm 1	PL	82%	83%
	PLP	-	60%
9 w. Farm 2	C	-	0%
	PL	-	78%
	PLP	-	68%

Conclusions

In the conditions of this study, this new vaccine has proved safe and showed seroconversion against a control group.

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Field evaluation of PCV2 dynamic infection and lung damage of two ready-to-use PCV2-*M. hyopneumoniae* vaccines

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Introduction

Development of ready-to-use PCV2 and *M. hyopneumonieae* vaccines have facilitated farm management as well as the control of these two diseases. However, in field conditions there are few studies in which the protection provided by them are measured at same time.

The objective of this study was to compare the PCV2 viremia and antibody response, as well as *M. hyopneumoniae* compatible lung lesions between the two ready-to-use vaccines and a group of unvaccinated pigs.

Materials and Methods

The study was done in a commercial farm in which PCV2 Systemic Disease had previously been diagnosed in fattening pigs.

When piglets were 26 days old, all of them were identified and allocated into three groups (A, B and C with 207 to 226 piglets/group), according to their weight and number of parturitions. That day, piglets in Group A were vaccinated with Porcilis® PCV M Hyo, in the Group B with Suvaxyn® Circo+MH RTU and the Group C was used as a control.

From each vaccinated group 40 random piglets and 20 from the unvaccinated one were identified with an extra tag. From each one of these animals, a serum sample was taken at 26, 42, 63, 84, 105, 133 and 159 days of live. Serum samples were analyzed in pools of 5 to detect and quantify PCV2 viremia and individually to measure PCV2 antibodies.

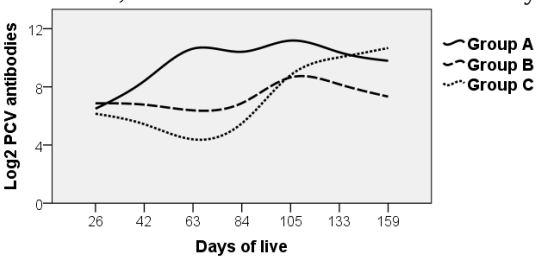
Lung check was performed at slaughterhouse for a subset of pigs in each group (84 to 89 pigs per group). Lung scoring was performed individually according the method established by Madec (1) normalizing the values according the relative volume of each lobe (2). Thus, for each pig it was calculated an index between 0 and 4. In addition, if any lobe had a scar, an extra point was added to that index (3).

Friedman test of repeated measures was employed to compare differences in the PCV2 antibodies in each group between consecutive visits. To compare the lung index between the three groups, it was used the Kruskal-Wallis test, with Dunn *post hoc* and Bonferroni correction.

Results and Discussion

In the Group C PCV2 viremia was detected since 84 days, with a peak between 84 and 105 days (4/4 positive pools with 2.66 to 3.28 $x10^4$ copies/ml). In the Group B, only one positive pool was detected at 84 and 105 days (9.57 and 9.66 $x10^2$ copies/ml), in contrast, in the Group A viremia never was detected.

PCV2 antibodies dynamic is showed in the figure. In Group A piglets seroconverted between 42 and 63 days; in the others, this occurred between 84 and 105 days.



Lung index was 0.96±0.63 for Group A, 1.10±0.61 for Group B and 1.18±0.60 for Group C. Pigs from Group A showed a statistically better index than pigs from Group C (p=0.04). The rests of comparisons did not show any difference.

Conclusions

Both vaccines were able to reduce the proportion of PCV2 infected pigs and the viremia. In addition, both vaccines reduced *M. hyopneumoniae* compatible lesions, but compared with unvaccinated pigs, only the vaccine Porcilis® PCV M Hyo improved significantly this parameter. This vaccine also induced a PCV2 seroconversion after its administration.

Acknowledgments

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Glaesserella parasuis is highly prevalent in backyard pigs in Southern brazil

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Introduction

Glaesserella parasuis is an important pathogen of pigs that cause an inflammatory disorder known as Glässer's disease. *G. parasuis* causes high morbidity and mortality leading to significant economic losses especially in pigs at the nursery phase. Serological diagnostic of *G. parasuis* can be performed by an ELISA based on the OppA protein (1), which is commercialized by BioCheck company (USA). Recently, our group developed and validated a new indirect ELISA based on a recombinant Periplasmic Ferric binding Protein A (FbpA) capable of detecting pig anti-*G. parasuis* antibodies with high specificity and sensibility. Here, using this new ELISA, we investigated the prevalence of IgGs against *G. parasuis* in backyard pigs from Southern Brazil.

Materials and Methods

Serum samples were collected from pigs in Rio Grande do Sul - RS (southern region of Brazil). Backyard pig production is distributed in approximately 130,000 farms with an estimated population of 915,000 animals (2). Sampling was carried out within the biannual surveillance for classical swine fever virus (CSFV) performed in RS by the official veterinary office (Secretaria Estadual da Agricultura, Pecuária e Irrigação [SEAPI-RS]), and attended the following criteria: first, the number of farms was defined aiming to detect at least one positive farm using a confidence level of 95% and an expected prevalence of CSFV of 1%. Second, the number of samples collected at each farm was defined to detect at least one positive pig using a confidence level of 95% and an expected prevalence of 10% within the farm. With these parameters, 713 sera were collected from 196 pig farms distributed into 142 different municipalities in 2014. The presence of anti-*G. parasuis* IgG was assessed by an *in-house* indirect ELISA based on recombinant FbpA protein, as previously described **(3)**. Briefly, ELISA plates were coated with FbpA (5 µg/well) in carbonate buffer (pH 9.6) and the wells blocked with 5% (v/v) skim milk (Sigma Aldrich, USA) diluted in PBS-T. Pig test serum (diluted 1:100) was added to the plates and incubated at 37°C for 1 h. After PBS-T washing, peroxidase-conjugated rabbit anti-pig IgG (Sigma Aldrich, USA) diluted 1:10,000 was incubated as indicated above. Subsequently the TMB substrate solution (Sigma Aldrich, USA) was added and the plates were read at 450 nm using a Synergy HI plate reader. The *cut-off* point of this ELISA was adjusted to an optical density twice higher than the negative control mean.

Results

We found 441 positive samples for anti-FbpA IgG. The overall seroprevalence for *G. parasuis* was 61.85% (95% confidence interval, 58.2 to 65.4%). Out of 142 municipalities analyzed, 88 (61.97%) had at least one pig farm positive for *G. parasuis.*

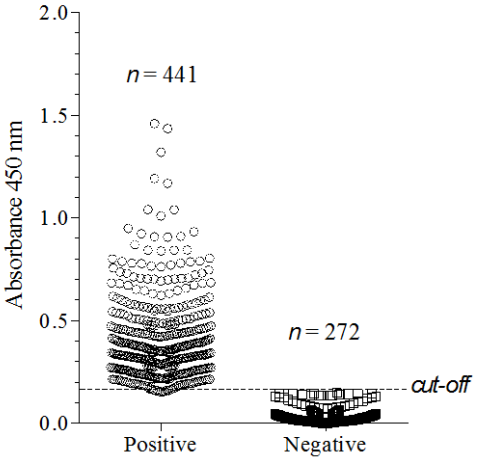

Figure 1. Prevalence of anti-FbpA antibodies in 713 pig sera. Results are depicted as absorbance values at λ = 450 nm.

Conclusions and Discussion

Here we demonstrated a high prevalence of backyard pigs serologically positive for *G. parasuis* distributed in all regions where intensive pig production is carried out in RS. Because vaccination of backyard pigs against *G. parasuis* is not frequently performed, we can assume that this high positivity highlights an endemic infection process in the population studied which might represent a risk for the commercial pig farms.

Acknowledgments

This work was supported by AFK Imunotech.

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Development of a new serological diagnostic test for *Lawsonia intracellularis* infection

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Introduction

Lawsonia intracellularis is an obligate intracellular bacterium of pigs associated with two types of clinical manifestation: (i) a chronic form described as porcine proliferative enteropathy (PPE) commonly observed in pigs under 4 months of age, and (ii) an acute form known as proliferative hemorrhagic enteritis (PHE) usually found in pigs older than 4 months. The subclinical form of the infection is predominantly found in the field and is characterized by decreased growth rates, variation in pig size and shedding of *L. intracellularis* in feces (1). The serological diagnosis of *L. intracellularis* is carried out by immunofluorescent antibody test (IFAT) (2), immunoperoxidase monolayer assay (IPMA) (3) or blocking ELISA (4), these techniques differ in terms of sensitivity and specificity. Here, we present a novel, flow cytometry (FC)-based methodology to assess pig antibodies against *L. intracellularis* which can revolutionize the serologic diagnosis of this pathogen.

Materials and Methods

To validate the FC method to detect pig antibodies (IgG) against *L. intracellularis* (Li), viable bacteria from a commercial vaccine (Enterisol Ileitis attenuated vaccine, Boehringer Ingelheim, USA) were used to adjust the cytometer optical parameters (forward-side scatter – FSC and side-side scatter – SSC). The lyophilized vaccine was reconstituted using 50 mL of sterile water and total bacteria from 1 mL were counted using the cytometer (BD FACSVerse™ - BD Biosciences, USA) equipped with volumetric counter. Then, 1×10^5 Li were incubated with pig sera diluted 1:100 in FACs buffer (Phosphate buffer + 1% of bovine serum albumin, pH 7,4) for 20 min at room temperature (RT). The bacteria were washed 3 times and coupled IgGs were detected using 50 ng of phycoerythrin labeled (PE) goat anti-pig IgG (Santa Cruz Biotechnology, USA). Finally, the bacteria were washed again, stained with Syto™ 24 (Thermofisher, USA) and acquired. The data were analyzed using BD FACSuite™ software (BD Biosciences, USA) and the strategy illustrated in figure 1. A panel of sera collected from specific pathogen free pigs (n=40), conventional pigs molecularly negative to Li (n=20), vaccinated (n=20) (Porcilis® Ileitis, MSD) or experimentally infected with Li (n=20) were used. The area under the curve (AUC) analysis was used to evaluate the test accuracy.

Results

L. intracellularis obtained from Enterisol Ileitis vaccine was easily detected and quantified by FC (Fig. 1A). Using negative pig sera (n=60) incubated with Li^{Syto+} we observed a low percentage of bacteria gated in R2 with associated IgG (3.2 ± 1.3%) (Fig. 1C). In contrast, the positive sera (n=40) from infected or vaccinated pigs were highly capable to recognize the bacteria (62.8 ± 22.8%, Fig. 1D). The AUC of this test was 1 (95% confidence interval, $p < 0.0001$). Using a cut-off of 11.7% the assay showed sensitivity and specificity of 100%, respectively.

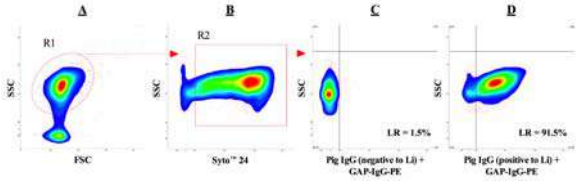

Figure 1. Detection of pig IgG against *L. intracellularis* by flow cytometry. **A**: Flow cytometric Contour Plot (hybrid) of Li using FSC *versus* SSC parameters (R1: gate for Li). **B**: Li with stained DNA (Syto™ 24, green) were gated in R2 (studied population). **C**: Li^{Syto+} incubated with negative serum from conventional pig and incubated with goat-anti pig IgG PE labelled (GAP-IgG-PE). **D**: Li^{Syto+} incubated with positive serum from infected pig and incubated with GAP-IgG-PE. The percentage of IgGs recognition is illustrated in the low right quadrant (LR) of the Contour Plots C and D.

Conclusions and Discussion

Here we present a new method to detect antibodies against *L. intracellularis*. We used unfixed life bacteria for the FC analysis which allows the detection of antibodies against conformational epitopes displayed on the bacteria surface and this likely contributed to the high sensitivity and specificity observed, making FC use rational in the serological diagnosis of *L. intracellularis*. The *L. intracellularis* source for this diagnostic assay can be purchased commercially (Enterisol Ileitis vaccine) making this technique reproducible among laboratories.

Acknowledgments

AFK Imunotech kindly supplied the reagents used.

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A Tbpb^{Y167A} based vaccine: the first vaccine capable of preventing *Glaesserella parasuis* colonization in pigs

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Introduction

The major entry point for many pathogens that cause disease in pigs occurs at the respiratory or gastrointestinal mucosal surfaces. To cause disease, *Glaesserella parasuis* overcomes the innate mucosal system and disseminates to distant systemic sites by entering into the blood stream and produces a severe inflammatory disease in young piglets, known as Glässer's disease (GD). This disease can be prevented by the use of a subunit vaccine based on TbpB^{Y167A}, which elicits a functional antibody response (1-3). Protective immunity against mucosal pathogens require novel vaccine strategies to induce mucosal immune responses in the entering site of the invading pathogen. Therefore, in the present study we developed a novel vaccine for application on the oral mucosal of pigs using a needle-free device.

Materials and Methods

A microparticle TbpB^{Y167A} based vaccine was generated by coacervation and stabilization of poly [di(carboxylatophenoxy)-phosphazene] using NaCl and CaCl2, respectively (4). Vaccine sterility was assessed as recommended by European Pharmacopeia and safety and immunogenicity in a total of ten 7-week-old C57/BL6 mice. A total of 10 commercial-hybrid Large White × Landrace pigs born from positive sows for *G. parasuis* were randomly divided in two groups: MPv-TbpB (n=6) and MPv-PBS (n=4). At 7 and 21 days old, piglets were immunized directly on the oral mucosa (0.2 mL, jugal position) using a needle free device (Comfort-in™ Needle Free Injector Kit). Serum, oral and respiratory swabs were collected before immunizations and challenge, to assess the titres of IgG(total), IgG1, IgG2, IgA, s-IgA and IgM by an indirect *in-house* ELISA based on bio-TbpB^{Y167A} captured on a streptavidin plate. At day 42, all piglets were intranasally challenged using 4×10^7 CFU of *G. parasuis* SV7, 174 strain. Following the challenge, clinical (twice a day), pathological and microbiological analysis were performed for 15 days. The animal experiments reviewed and approved by the Animal Care Committee of the University of Calgary (AC17-0153).

Results

The MPv-TbpB was safe and capable of inducing IgG titres (8.960 ± 4.213) in C57/BL6 mice 14 days after the second immunization. In pigs, vaccinated animals harbored significantly higher levels of anti (α)-TbpB^{Y167A} antibodies (IgA, s-IgA, IgM, IgG, IgG1 and IgG2) prior to challenge compared to the control pigs as illustrated in the Figure 1 a-g.

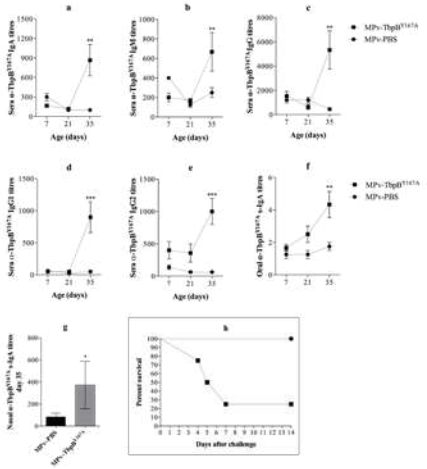

Figure 1. Immunoglobulins profile induced by MPv-TbpB^{Y167A} in pigs and clinical survival rates.

Before the challenge, nasal swabs from all vaccinated pigs were negative for *G. parasuis.* In contrast, 3 of 4 control animals were positive. After the challenge 3 of 4 control pigs died due to GD. In contrast, all vaccinated animals survived challenge (Fig. 1h) and lacked clinical signs (except for some sneezing) and pathological lesions.

Conclusions and Discussion

Glässer's disease can be prevented by vaccination by the TbpB^{Y167A} protein which is one of the more promising antigens to include in a broad-spectrum vaccine for GD. In this study we demonstrated that direct mucosal immunization can induce mucosal sterility against *G. parasuis*, opening a new avenue for eradication of this pathogen affecting pig production.

Acknowledgments

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Comparison of two different vaccination schemes for IAV-S control

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Introduction

The Influenza virus type A (IAV-S), is an endemic infection responsible for causing a respiratory disease, with a significant morbidity as well as a very important worldwide economic impact of US $3.23 per pig[1]. During the past, the only way to try to induce a controlled immune response in pigs was using autogenous vaccines, which works well but only against homologous virus challenge. A novel Live Attenuated Influenza Vaccine (LAIV), Ingelvac Provenza™ is now available in the Mexican market to protect against multiple IAV-S strains. The technology behind this vaccine is the truncation of the NS-1 protein, which provides the production of interferon γ. The aim of this study is to understand the positive impact on expected variables, when use a LAIV for disease control in a parity one flow considered with high IAV-S challenges.

Materials and Methods

The study was conducted in a 2,400 multi-site system, positive to IAV-S, PRRS and Mhp. located in the central region of Mexico. An autogenous vaccine from a local laboratory was used in the baseline vaccination protocol for gilts, sows and piglets. The autogenous vaccine was continuously updated with the circulating IVA-S subtypes. The piglets (including gilts) were vaccinated two times in the nursery, at five and seven weeks of age; gilts were re-vaccinated two more times during acclimation. The sows received the autogenous vaccine at 11 and 13 weeks of gestation, and this was not changed during the trial. Before the study was initiated, a sampling monitoring using oral fluids (OF), was established in the nursery, to understand the IAV-S dynamic of infection and analyzed by real time PCR (QuantStudio 5). The results showed the exposure to IAV-St at 7 weeks of age, and had a strong relation with the pig performance observed such as reduction of daily weight gain and high mortality impact. The region were the farm is located has a range of temperature between 3°C to 21°C during the year, and for that reason the groups were selected taken in mind the season. The pigs of the autogenous group were born between December 2017 and May 2018, and the LAIV group were born between May 2018 and December 2018. On week 22 of 2018, the study began following the protocol, vaccinating pigs from 3 to 5 days of age, administered via the intranasal route in a single 1 ml dose[2, 3, 4]. Diagnostic monitoring on the LAIV group were established, using biweekly OF in three previous selected barns, by using RT-PCR for IAV-S and PRRSV. A total of 26 pig batches were observed during 6 months, and production parameters from wean to finish, such as mortality rate, average daily weight gain (ADWG), and Feed Conversion Rate (FCR), were analyzed using a two sample t-test (SAS® Enterprise Guide 4.3, Cary, NC, USA), and compared with 24 groups from the baseline (historical information).

Results

Mortality rate, ADWG and FCR results are shown in Table 1. Mortality rate and ADWG in the nursery were significantly improved in the LAIV group compared to the autogenous group. ($p \leq 0.05$). Mortality was reduced from 13.08 % in the autogenous group to 8.9 % in the LAIV group.

Conclusions and Discussion

The groups vaccinated with Ingelvac Provenza™ performed better comparing with the autogenous group, decreasing wean to finish mortality to 8.9% (4.18% less than autogenous group), and increasing ADWG to 0.741 Kg (+0.045 Kg/day). The diagnostic results on the LAIV vaccine group showed the exposure to both IAV-S strains, H1N1 and H3N2 during nursery and finishing stages, and endemic wild PRRS strain circulation in all weeks of study. Although we found diagnostic results that showed the presence of wild type IAV-S, Ingelvac Provenza™ demonstrated significant efficacy and protection.

Table 1. Mean results from Autogenous and LAIV on mortality rate, ADWG and FCR.

Group	# of pigs	% Mort.	ADWG Kg	FCR
Autogenous	21,938	13.08	0.696	2.33
Provenza™	24,653	8.9	0.741	2.33
Difference		*4.18*	*0.045*	*0*
*p- value**		*0.000**	*0.01**	*0.957*

*Statistical difference between groups ($p \leq 0.05$).

An in-depth analysis must been done to further understand the impact of Ingelvac Provenza™ on reduction of excretion and antibiotic reduction on bacteria control.

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Decreasing sow mortality after vaccination against *Clostridium novyi*

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Introduction

Clostridium novyi has been shown to be a pathogen which can cause sudden death in breeding and fattening pigs, many cases of *Clostridium novyi* having been reported from different countries (1,2,3). Furthermore, using different diagnostic techniques, more cases of *Clostridium novyi* are confirmed in the samples from sows that have suffered sudden death (4). The aim of this study was to evaluate the impact of SUISENG®, (a commercial vaccine containing fimbriae and toxoids against *Escherichia coli*, *Clostridium perfringens* type C and *Clostridium novyi*) on the sow mortality rate after implementing a rolling vaccine program on a commercial farm in 2017.

Materials and Methods

A commercial farm with 3,900 sows, with a high mortality rate in South Korea, was enrolled in this trial. After the necropsy examination and laboratory diagnosis, *Clostridium novyi* and its α toxin were isolated. Finally, the entire farm started vaccinating and revaccinating following the manufacturer's instructions. To evaluate the efficacy of SUISENG®, the sow mortality rate was compared between two different periods (A and B). Period A encompassed 2015 and 2016, when no *Clostridium novyi* vaccines where used. On the other hand, Period B included 2017 and 2018, when the farm started vaccination with SUISENG®. These periods where compared using a Wilcoxon test.

Results

Based on the historical data, the monthly mortality rate significantly decreased from 0.85% to 0.49% (*p-value; 0.0006)* when Period A and Period B were compared *(Figure 1)*. This represents a reduction of 42% (Figure 2).

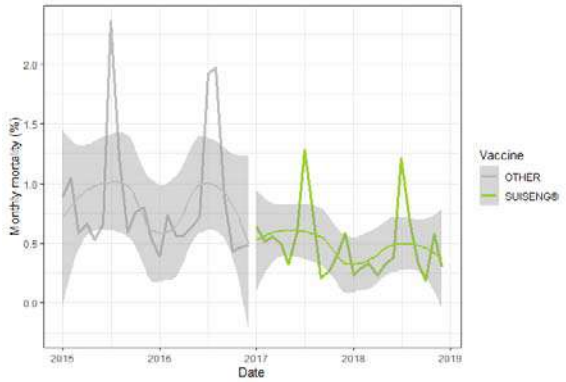

Figure 1. Evolution of the monthly mortality rate before and after vaccination.

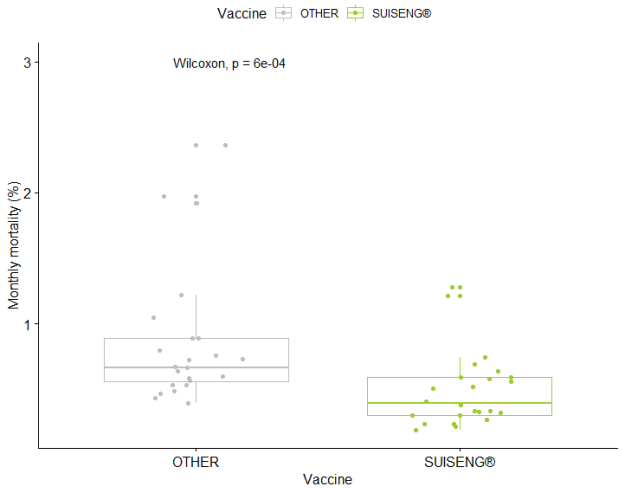

Figure 2. Average sow mortality rate in period A and period B.

After two years using SUISENG® (2017 and 2018), the reduction in mortality was up to 239 sows when compared with the period before vaccination (2015 and 2016). This represents a valuable improvement if we take into consideration the average cost of a gilt (350€) (5).

Conclusions and Discussion

The decrease in mortality after implementing SUISENG® vaccination was significant. Therefore, the connection between decrease in mortality and the use of vaccine indicates that immunization against *Clostridium novyi* could be responsible for a significant reduction in the percentage of sow mortality cases. The ultimate objective of this reduction is to maximize productivity whilst maintaining a regular production schedule. Further studies should be performed to evaluate the return on investment of sow vaccination against sudden death.

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Serological monitoring as a tool for health management in swine herds

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Introduction

The health status of pig populations, together with the presence, severity and impact of disease, varies from farm to farm and is multi-factorial in nature.

Routine and consistent monitoring of infectious diseases in a production system is an effective strategy to proactively manage their impact in pig health and production. To that end, the use of ELISA in monitoring programs is a rapid, inexpensive and easily automated diagnostic method.

This study describes the implementation of serological monitoring programs for salmonellosis, swine influenza, porcine pleuropneumonia and enzootic pneumonia in swine farms.

Materials and Methods

Thirteen multi-site herds located in Brasil were selected for this study. In each herd, 20 serum samples were collected monthly from December 2016 to October 2019 in each of the following groups: gilts (26-weeks old), sows (1-week before farrowing), growers (10-weeks old) and finishing pigs (20-weeks old). Serum samples were tested with the following ELISA kits according to the manufacturer's recommendations: Swine Influenza Ab Test, *Mhyo* Ab Test, APP-ApxIV Ab Test and Swine Salmonella Ab Test [IDEXX Laboratories, Inc., Westbrook ME, USA].

Results and Discussion

Monitoring of swine salmonella showed changes in average S/P values over time by age group. An increase in S/P values of sows in autumn 2018 (Figure 1) and finishing pigs in winter 2018 was recorded.

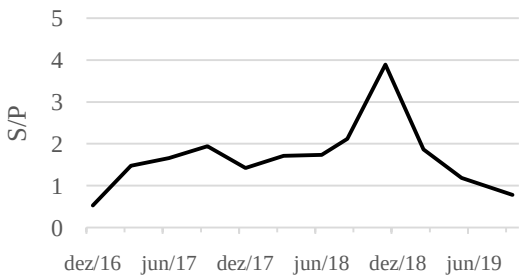
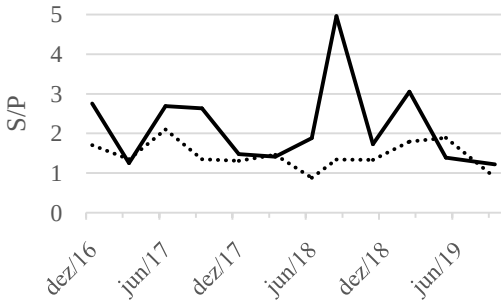


Figure 1. Salmonella monitoring in sows. Average S/P values over time.

Swine influenza monitoring showed that finishing pigs in the studies herds had seroconverted by 20 weeks of age (average S/N value < 0.6). Other animal groups tested remained negative throughout the studied period.

Actinobacillus pleuropneumoniae (App) monitoring showed that swine pleuropneumonia was stable over time in sows, gilts and growers. However, an increase in average S/P values was observed in finishing pigs in autumn 2017 and in summer-autumn period of 2018, indicating active App infections during the growing period for those batches of pigs.

Figure 2. *Mycoplasma hyopneumoniae* (*M.hyo*) monitoring in sows (dotted line) and gilts (solid line). Average S/P values over time.



Changes in average S/P values for *M.hyo* were recorded for finishing pigs before and after summer 2018 (data not shown). Whereas *M.hyo* S/P values remained stable in sows, instability was observed in gilts, suggesting a recent past infection and the need of adapting *M.hyo* acclimation practices accordingly (Figure 2).

Conclusions

In this study, the implementation of monitoring programs for four economically important diseases of pigs provided useful information on the presence of antibodies and seroconversion, helping to detect new infections, evaluate vaccination response and optimize health and management practices to stabilize the immunity of the herd.

The implementation of monthly serological monitoring programs remains a valuable tool to understand health status, immunity and infection dynamics, contributing to the optimization of pig production in monitored herds.

Comparison of two multivalent respiratory vaccines in field Mexican conditions

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Introduction

Porcine respiratory disease complex is a multifactorial syndrome involving viral and bacterial pathogens as well as environment and management (1). Multivalent vaccines allow to induce immunity against different pathogens by a limited number of injections. Objective of this trial was to compare two multivalent respiratory vaccines in field Mexican conditions.

Materials and Methods

The trial started in a farm with 200 sows (2 weeks batch management) till the end of nursery. This farm had history of respiratory diseases due to *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae.* Then trial pigs were moved to an experimental center till finishing. Two successive replicates were included in the trial (I and II). For each replicate, 10 pregnant sows were randomly allocated to tested group (T receiving the tested vaccine) or control group (C receiving the control vaccine), according to parity ranging from 0 to 6 (5 sows per group and per replicate). Sows were vaccinated twice, 2 weeks apart during last third of pregnancy. Piglets issued from each sow were vaccinated twice with the same vaccine around 3 and 5 weeks of age. Pigs were housed in collective pens per group in nursery and fattening units.The tested vaccine comprised bacterins and toxoids of *Pasteurella multocida, Bordetella bronchiseptica, Actinobacillus pleuropneumoniae* and bacterins of *Streptococcus suis, Haemophilus parasuis Mycoplasma hyopneumoniae,* (Suigen® Donoban® 10, Virbac). The control vaccine comprised bacterins of *Pasteurella multocida, Bordetella bronchiseptica, Haemophilus parasuis, Mycoplasma hyopneumoniae, Mannheimia haemolytica* and toxoid of *Pasteurella multocida* (Rhinanvac®, Syva). Both vaccines were injected by intramuscular route according to label (2 ml in sows and piglets for C vaccine, 2 ml in sows and 1.5 ml in piglets for T vaccine, per injection). Respiratory symptoms were regularly checked in nursery and fattening units by standardized scoring. Pigs were individually weighed at finishing. For each slaughtered pig, a global scoring of pneumonia lung lesions was recorded as 0 (no lesions), 1 (lesions area≤ 25% of total lungs area), 2 (lesions area between 26% and 50% of total lungs area), 3 (lesions area between 51% and 75% of total lungs area) or 4 (lesions area > 75% of total lungs area). Categorical data were compared between groups by Fisher exact test or Cochran-Mantel-Haenszel test stratified on the replicate. Body weights were analysed by two-way analysis of variance (group and replicate).

Results

No side effects were observed after vaccines injection both in sows and piglets. A total of 175 and 170 pigs were respectively followed in nursery and fattening. Lung lesions were scored on 152 out of the 170 slaughtered pigs. Morbidity and mortality rates were numerically lower in T than in C group, difference being close to statistical significance for morbidity in nursery (p=0.08, Table 1). Morbidity rates were higher in 1st replicate, possibly in link with winter season. Final body weights were not significantly different between groups, though mean value was 4 kg higher for T group in 1st replicate (Table 2). Weights were significantly higher in 1st than in 2nd replicate (p=0.0004), probably due to older age at slaughtering. Lung lesions scores were significantly lower in T group (Table 3).

Table 1. Respiratory morbidity and mortality rates.

	Morbidity		Mortality	
	Nursery	Fattening	Nursery	Fattening
T	6.7%	0%	1.1%	0%
C	15.1%	1.2%	2.3%	1.2%

Table 2. Final body weights (mean ± SD as kg).

Replicate	I	II	I + II
T	99.5±14.3	103.1±6.5	101.4±11.2
C	95.5±14.8	104.9±8.5	100.0±13.0

Table 3. Distribution of lung lesions scores.

Score	0	1	2	3	4
T*	44.3%	34.2%	11.4%	10.1%	0%
C**	19.2%	39.7%	21.9%	12.3%	6.8%

***:Significant difference between groups (p=0.0005)

Discussion and conclusion

Vaccination program including sows and piglets allowed to control respiratory diseases. T vaccine efficacy has been previously described in Vietnam (2) and in the Philippines (3). Lower lung lesions scores with T vaccine in the present study may be due to wider antigens composition, particularly including *Actinobacillus pleuropneumoniae* and *Str. Suis.*

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Effect of an oral live vaccine against *Lawsonia intracellularis* on the performance of pigs at a production system in Mexico

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Introduction

Porcine reproductive enteropathy (PPE) is caused by *Lawsonia intracellularis* and has a considerable economic impact on swine production worldwide, affecting pigs during the grow-finish period[1]. The objective of this study was to evaluate the impact of applying the only live oral vaccine against *Lawsonia intracellularis* (Enterisol® Ileitis) on pig performance in a production system in Mexico.

Materials and Methods

This study was performed in an 1800 multi-site sow farm located in Jalisco, Mexico. The sow farm works as a 3-week batch production system. At 24 days of age, piglets are weaned and sent to a wean to finish site. The production system decided to implement a vaccination program with an oral live vaccine (Enterisol® Ileitis), after constantly observing lesions related to *Lawsonia intracellularis* at the slaughterhouse, and exposure to *Lawsonia* was confirmed by serology. In this study, a comparison was made before and after implementation of vaccination. A total of, 39975 pigs were included and divided into two groups: Group A with 25921 animals (batch 1-7) without ileitis vaccine and Group B with 13794 (batch 8-10) vaccinated animals, given a 2 ml oral dose of Enterisol® Ileitis prior to wean. For this study, it was decided to evaluate the following parameters: average daily weight gain (ADWG), feed conversion rate (FCR), market weight (MW) and market age (MA). These parameters were analyzed by the Two-sample T-test method (Minitab 17) and an economic analysis was done using BECAL (Boehringer Ingelheim Economic Calculator).

Results

After the implementation of Enterisol® Ileitis, we observed a numerical difference in all the parameters evaluated and a statistical difference in ADWG ($p<0.05$), when comparing the vaccinated with that non-vaccinated groups (Table 1).

Table 1. Parameters evaluated.

Parameters	Group A	Group B
ADWG	0.699[a]	0.730[b]
FCR	2.19	2.11
MA	168.8	163.5
MW	108.1	107.9

(a,b) Different letters indicate differences at $p < 0.05$.

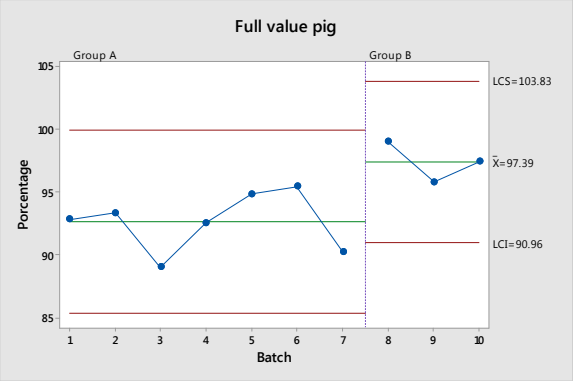

Figure 1. Full value pig.

Conclusions and Discussion

Group B (vaccinated) demonstrated a better production performance compared to Group A (non-vaccinated). The difference in ADWG and FCR was 0.031 kg/day[4] more and 0.08 less, respectively, which represent a gain of $2.32 and $3.61/pig, respectively. Using BECAL we obtained a ROI 5.61:1.[1-2]

In addition, more pigs in Group B reached the established weight and age comparing with pigs in Group A. (figure 1).

In this study, we observed that using a live oral ileitis vaccine (Enterisol® Ileitis) it was possible to improve pig performance and obtain a more efficient production system[3-4]

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Evaluation of the productive performance of pigs with the use of a trivalent vaccine (PRRS MLV/PCV2/ *Mycoplasma hyopneumoniae*) in a commercial farm in Mexico

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Introduction

The infection with the reproductive and respiratory virus (vPRRS) remains one of the diseases with the greatest economic and productive impact on pig production system worldwide[1]. Veterinarian and producers are constantly looking for the best strategies to control this virus. The use of MLV vaccine as part of the strategies has improved the performance and mortality losses generated by PRRS. The objective of this study is to evaluate the productive performance of pigs from weaning to finishing, vaccinated with 3FLEX® (PRRS MLV/PCV2/ *Mycoplasma hyopneumoniae*), in a farm in Mexico.

Materials and Methods

This study was performed in an 1800 multi-site sow farm located in Jalisco, Mexico. The sow farm works as a 3-week batch production system. Pigs were treated under two different vaccination protocols. The farm is positive for PRRSv, *Mycoplasma hyopneumoniae*, Porcine Circovirus type 2 and Influenza type A. A total of 9,723 pigs were included in this study and divided as follows: 4,789 pigs belonging to "Group A" received a single dose of 2 ml IM of FLEXcombo® (PCV2+*Mycoplasma hyopneumoniae*) at 21 days of age. The other group ("Group B") that included 4,934 pigs, received a single dose of 3FLEX® (PRRS MLV + PCV2 + *Mycoplasma hyopneumoniae*) at 21 days of age. In this study, a statistical analysis (X^2) of % weaning mortality was made between treated and untreated groups (Table 2), which could not be extended to other productive parameters, because these were measured at the group level and not individually or per/pen. Through BECAL (Boehringer Ingelheim Economic Calculator), the profitability of the new vaccination program at the end of the study was calculated, comparing the performance of both groups.

Results

Average daily weight gain (ADWG), feed conversion rate (FCR), final weight and % weaning mortality were selected as productive parameters for measurement. The results are shown in Table 1.

Table 1. Comparison of two vaccination protocols.

Groups	%Mort. Weaning	ADWG	FCR	Final weight
A	3.01	0.695	2.30	111.64
B	1.99	0.702	2.21	116.95
Diff.	-1.02	+0.007	-0.09	+5.31

Table 2. Comparison of mortality % Group A vs. Group B at weaning stage.

Category	Group A	Group B	Mortality %*
Alive	4645	4836	3.01^b
Dead	144	98	1.99^a

*(a,b) Different letters indicate differences at $p < 0.05$

A numerical difference was observed in all the parameters evaluated. The difference observed in mortality % of Group B vs. Group A was 1.02% ($p<0.05$), which is equivalent to a gain of 0.94$ for each pig. The difference observed in ADWG and FCR of Group B vs Group A was 0.007 kg/day and -0.09, which is equivalent to a gain of 0.72$ and 3.43$ respectively per pig

Conclusions and Discussion

After the implementation of 3FLEX® in piglets, the control of PRRS was improved by observing a reduction in mortality and improvement in productivity in general. Using BECAL, we obtained a ROI 8.72: 1[2,3,4]

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Shedding of a live attenuated influenza A virus vaccine in pigs under field conditions

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Introduction

Influenza A virus (IAV) is one of the most important respiratory diseases affecting swine health. Though H1N1, H1N2 and H3N2 are endemic subtypes found in pigs, there is significant diversity within the IAV subtypes[1]. Antigenic drift and shift result in IAV changes that limit the efficacy of killed vaccines currently used in pigs. A new live attenuated influenza vaccine (Ingelvac Provenza™) is now available in the U.S market and could be an important tool to control IAV in swine[2]. Because the vaccine is live and can replicate in vaccinated animals, it is important to assess how much and for how long the vaccine virus can be shed and whether it can transmit to other pigs.

Therefore, the objectives of this study were to assess the onset and duration of vaccine virus shedding, investigate the ability of vaccine virus to transmit from vaccinated to unvaccinated pigs and evaluate whether the vaccine virus can be aerosolized and detected in the environment

Materials and Methods

Five farrowing rooms of a 5,200 sow farm that vaccinated pigs at processing with Ingelvac Provenza were selected for the study. Thirty-three litters were selected within three farrowing rooms following a pre-determined spatial pattern. Within vaccinated litters (n=21) six pigs per litter were tagged, three of the pigs were left unvaccinated while the rest of the pigs in the litter were vaccinated to assess direct contact transmission to the unvaccinated pigs. There were 12 litters left unvaccinated (4 litters/room) and three pigs within these litters were tagged to assess transmission by indirect contact.

Nasal swabs were obtained from all tagged pigs before vaccination and at 1, 2, 3, 4, 6, 8 and 12 days post vaccination (DPV). Udder wipes from lactating sows and surface wipes of the farrowing crates were also sampled on the same days. Air samples and samples representing surface deposition of airborne particles were collected immediately after vaccination of the entire room and at 1, 2, 3, 4, 6 and 8 DPV. Nasal swabs were tested in pools of three and pooled within litters. Samples were screened for IAV using a vaccine strain specific rRT-PCR. Samples with cycle threshold (ct) ≤ 38 were considered positive and ct >38 considered negative.

Results

One hundred percent (21/21) of the vaccinated litters tested vaccine virus positive 1 DPV. At 6 DPV, 8% (2/21) of the litters tested positive which was the last time when vaccine virus was detected in vaccinated litters. In contrast, only five of the non-vaccinated pigs tested positive during the course of the study. Seventy-six percent (16 out of 21) of udder wipes in vaccinated litters tested positive at 1 DPV and until 12 DPV (1 out of 21). Eight percent (1 out of 12) of udder wipes in the non-vaccinated litters tested positive at 6 and 12 DPV, respectively.

In addition, low levels of LAIV RNA (PCR ct values ranging between 33 and 38) were detected in all air specimens collected on the day of vaccination and until 6 DPV (3/10 (30%)). Samples from surfaces that represented the deposition of airborne particles had a 68% (17/25) positivity on the day of vaccination and it decreased gradually until 5% (1/18) at 8 DPV.

Conclusions and Discussion

In this study we found evidence of limited transmission of LAIV to susceptible, non-vaccinated, sentinel pigs with indirect contact to vaccinates but sharing the same air space and to non-vaccinated pigs in direct contact as vaccinated pigs. We concluded based on our results, that although some LAIV was detected in non-vaccinated pigs with direct contact and in non-vaccinated sentinel pigs having indirect contact that LAIV detection did not result in sustained shedding in the non-vaccinated pigs. There was limited shedding of LAIV into the air and environment. More research is needed to further understand the transmission dynamics of LAIV, and the implications that the use of LAIV could have on influenza control in swine farms.

Acknowledgments

This study was funded by Boehringer Ingelheim Vetmedica, Inc. Duluth, GA 30096, United States.

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Vaccine warming may lead to vaccine failure

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Introduction

Some inactivated vaccines require either warming up to room temperature when containing mineral oil (1, 2) or warming up to body temperature when containing carbopol squalene (1) before use, respectively without a given explanation or to decrease side effects. In the Netherlands that resulted in a more general approach to warm up all piglet vaccines before use including vaccines that do not need warming up like vaccines with carbomer adjuvants (1, 2). Over time cases of vaccine failure were reported related to warming up of vials containing vaccine in hot water. This study tries to define a safe and efficient procedure for warming up vaccines before use.

Material and Methods

The temperature evolution of the content of vials was registered over time when being warmed up in water of different starting temperatures. Before use the vials were stored in a refrigerator at 4-6 degrees Celsius (^{o}C)/ 39-43 degrees Fahrenheit (^{o}F). The HDPE vials contained 100 ml of phosphate buffered solution representing vaccine for this experiment. The volume of water used for warming up the vials was 3 liter (60^{o}C/ 140^{o}F) or 12 liter (30^{o}C/ 86^{o}F, 40^{o}C/ 104^{o}F).

To measure the temperature in the vials the rubber stopper was punctured with a needle. The puncture hole was then used to stick in the probe tip of the thermometer (ADE BBQ 1600 Food Thermometer) for a distance of about 5 cm. Temperatures were registered with 2 minutes intervals over a 20 minutes period starting immediately after putting the vials in a large volume of water. For each of the water temperatures, 6 vials were tested.

Results

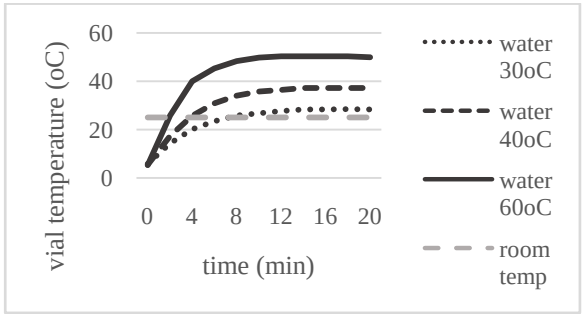

Figure 1 Temperature development over time (minutes) at the inside of 100 ml liquid filled HDPE vials in different temperatures of water.

Discussion and Conclusion

Warming up 100 ml vaccine vials up to a minimal room temperature of 15^{o}C/ 59^{o}F in an air temperature of 19^{o}C/ 66^{o}F up takes about 90 minutes (data not shown). Warming up vaccine in water may be an easier solution. Protein denaturation is temperature dependent and onset temperature of protein denaturation correlates well with body temperature (3). For this abstract a critical temperature for vaccine of 40^{o}C/ 104^{o}F and more is assumed. As almost all of the vaccines use a protein based antigen, it is likely that high vaccine temperatures affect the quality of the product leading to possible vaccine failures. The results show that in a warm water environment the temperature inside the vials rises to room temperatures (15-25^{o}C/ 59-77^{o}F) within 2 to 8 minutes. Vial content temperature becomes critical (above 40^{o}C/ 104^{o}F) within 4 minutes in a water environment of 60^{o}C/ 140^{o}F.

The conclusion is that the need for warming up inactivated vaccines is product dependent and is both efficient and safe to do this in a water environment of 30 to 40^{o}C/ 86 to 104^{o}F.

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Suvaxyn®CSF Marker, the first live marker commercial vaccine against Classical Swine Fever authorized in Europe and United States

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Introduction

Classical swine fever (CSF) is a highly contagious viral disease affecting pigs, which can lead to a tremendous socioeconomic impact. Within the European Union (EU) and in other areas with significant pig production, the disease is controlled by strict, mandatory control measures without prophylactic vaccination. However, emergency vaccination is usually foreseen and can be implemented in the case of a contingency. In this context, marker vaccines are preferred as they can reduce the impact on trade.

A potent live-attenuated Suvaxyn®CSF Marker vaccine was developed and licensed by the EMA and USDA. This vaccine combines advantages of current live C-strain vaccines (fast protection) and DIVA technology. The vaccine strain was also tested as oral marker showing that a bait marker vaccine for wild boar is feasible.

Materials and Methods

Vaccine virus. CP7_E2alf virus is a chimeric pestivirus whose coding sequences for the major envelope protein E2 of bovine viral diarrhea virus (BVDV- strain CP7) are replaced by E2 of the CSFV strain Alfort187. The vaccine has been evaluated both for intramuscular (IM) as well as oral vaccination as a bait vaccine.

Challenge virus. Highly virulent CSFV strain “Koslov” and moderately virulent CSF strain "Roesrath”

Animal experiments. Safety studies on pigs, pregnant sows, ruminants and rabbits; Minimum immunogenicity dose; Efficacy on domestic pigs after oral or IM vaccination; Onset of immunity after oral or IM Immunization; Duration of immunity after oral or IM Immunization; Transplacental protection after oral or IM immunization. Reversion to virulence; Dissemination; Virus shedding; Efficacy on wild boars after oral vaccination.

Results

Safety studies showed innocuousness and complete safety for target and non-target species, and no evidence was found for vaccine virus transmission to contact animals (1). Moreover, the virus did not cross the placental barrier in pregnant animals and did not affect the reproductive performance. In addition, the vaccine virus was not able to induce persistently infected offspring.

Efficacy was proven against highly virulent CSFV strains showing the same level of efficacy that the c-strain based vaccines. Onset of immunity was 14 days and duration of immunity was at least six months (2, 3, 4, 5, 6).

Recently, it was demonstrated that the vaccination in sows efficiently protected the offspring against transplacental transmission of moderately virulent CSFV (7).

In addition, Suvaxyn®CSF Marker vaccine can also prevent the establishment of postnatal persistence in the offspring. It was demonstrated that maternal derived antibodies from vaccinated sows can protect against postnatal persistence. Again, no differences were observed between the C-strain and the Suvaxyn®CSF Marker. This study demonstrates that a solid immunization of sows is of great importance in endemically infected areas.

Conclusions and Discussion

Taken together, Suvaxyn®CSF Marker (Zoetis) is the first live CSF marker vaccine suitability for emergency vaccination scenarios in countries with industrialized pig production that would allow deviations from the trade restrictions for vaccinated animals (8)

Acknowledgments

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Maternal derived antibodies (MDAs) induced by a Classical Swine Fever MLV marker vaccine prevent postnatal virus persistence in suckling pigs

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Introduction

Classical swine fever (CSF) is one of the most important viral diseases in pigs, having a large impact on the swine industry in many countries and being a continuous threat for the rest. While prophylactic vaccination is banned in the EU, mandatory vaccination is carried out in endemically affected countries (e.g. in Asia and South America) to reduce losses, with the goal of eradicating the disease.

Recently, the phenomenon of postnatal persistence has been described as having a negative impact on vaccination efficacy (1, 2). Postnatal persistence refers to the course of disease that can be induced in piglets in the early hours and days of their lives. It leads to constant shedding of CSF virus (CSFV), unspecific but mostly enteric clinical signs, and absence of specific antibody production. In the present study, we tested the hypothesis that effective vaccination of breeding sows and resulting in the transfer of maternal antibodies (MDAs) to their offspring, can prevent the induction of postnatal CSFV persistence.

Materials and Methods

Newborn piglets from sows vaccinated with either C-strain or Suvaxyn®CSF Marker (Zoetis) vaccine, as well as piglets from naïve sows were inoculated with the CSFV Catalonia strain 24 hours after birth. Upon weaning, the surviving piglets were vaccinated with the respective vaccine and challenged 28 days post vaccination with a CSFV strain of moderate virulence of German origin ("Rösrath").

Clinical signs and body temperatures were recorded during the study (3). Blood samples were collected regularly. At necropsy, tonsils, mandibular lymph nodes, salivary glands, lungs and spleens were collected.

Antibody detection was carried out by ELISA (IDEXX CSFV Ab ELISA) and neutralization peroxidase-linked antibody assays (NPLA) using CSFV strain "Alfort" and CSFV strain "Rösrath". Virus detection was performed by CSFV specific real time RT-PCR (4,5).

Results

In a nutshell, all naïve piglets developed postnatal persistence and showed extremely high viral genome loads and no detectable antibodies. These animals did not seroconvert upon active immunization. In contrast,

MDA positive piglets were completely protected against clinical infection. Moreover, these animals showed significantly lower genome loads and active antibody production. None of the MDA positive animals developed long-term persistence. No clinical signs were observed upon challenge.

Conclusions and Discussion

It was confirmed that:

- Vaccination is effective 5 weeks after birth in MDA positive piglets, even after early challenge.

- No significant differences were observed between Suvaxyn®CSF Marker vaccine and the C-strain vaccines.

- Vaccination of breeding sows can prevent the establishment of postnatal persistence in the offspring, which is very important in endemically infected areas.

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Predicted immune coverage of commercial PCV2 vaccines to contemporary Brazil PCV2 field strains

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Introduction

Immunization of pigs against Porcine Circovirus type 2 (PCV2) with commercial vaccines has become the common practice in swine farms across the world. However, PCV2 is genetically evolving rapidly using mechanisms such recombination and mutation, and the divergence of isolates is increasingly documented in the scientific literature. There is growing concern about commercial vaccines not providing enough cross-protection against new wild-type PCV2 isolates from different genotypes that may circulating in commercial farms.

The objective of this study was to compare T cell epitope content between PCV2 commercial vaccines and contemporary PCV2 field isolates from Brazil using swine leukocyte antigen (SLA) types common in commercial swine herds in order to predict immune coverage offered by vaccine strains to contemporary field strains from Brazil.

Materials and Methods

A total of 38 PCV2 sequences from recent Brazilian clinical cases associated with the virus were received. These PCV2 sequences were aligned to reference strains and it was determined that 11 belonged to PCV2b genotype, 19 were PCV2d genotype, and 3 sequences were incomplete.

We used PigMatrix to identify T cell epitopes in the proteomes of the contemporary PCV2 strains from Brazil and two commercial PCV2a-based vaccines (a baculovirus expressed ORF2 and a PCV1-PCV2a chimeric virus; cPCV2a) and an experimental combination cPCV2a-cPCV2b vaccine. Epitopes of vaccine and field strains predicted to bind common class I and class II SLA alleles were identified.

We then applied the immunoinformatics tool EpiCC (EpiVax) to compare epitopes of vaccine strains to these field isolates. EpiCC was used to evaluate and compare T cell epitope cross-conservation and determine an epitope-based relatedness (vaccines to field strains) EpiCC score.

Results

Epitope content and conservation between vaccine and field strains varied. While all vaccine strains provided broad coverage of the field strains including heterologous genotypes, none of the vaccines covered all T cell epitopes in the field strains. PCV2a based vaccine strains generally scored higher against PCV2a reference strains but were not identical. The

experimental combination PCV2a-PCV2b vaccine had, on average, the highest EpiCC

Conclusions and Discussion

PCV2 continues to evolve and EpiCC analysis provides a new tool to assess the possible impacts of virus genetic divergence on T cell epitope coverage of vaccine strains.

Given that viruses from multiple genotypes are currently and increasingly found and that several PCV2 strains may co-exist on commercial farms, a combination of PCV2a and PCV2b vaccine strains may be required to provide optimal coverage of current and future field isolates.

Porcine Circovirus Type 2 (PCV2) Serology Survey in Ten Brazilian Herds

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Introduction

PCV2-associated disease (PCVAD) has become one of the most economically important emerging diseases worldwide. PCV2 vaccination represents a success story to the pig industry globally as most pigs are vaccinated against PCV2. An outcome of the vaccination is the potential presence of maternal derived antibodies (MDA). The study objective was to evaluate PCV2 serology in young pigs, specifically MDA, among and within swine herds in Brazil.

Materials and Methods

Pigs (n=600) were sampled from 10 herds. Herds were selected based on the PCV2 vaccination status of gilts/sows: During pregnancy (sows were vaccinated for PCV2 at least once during gestation of their current litter and may have been vaccinated previously); During gilt development (sows were vaccinated for PCV2 during gilt development and may have been vaccinated previously); As piglet (sows were vaccinated for PCV2 as a piglet around the time of weaning or before); and Never vaccinated (sows have never been vaccinated for PCV2). Within each herd, two age categories of piglets were sampled: 3 ± 1 days of age and 21 ± 3 days of age; all piglets were sampled prior to piglet vaccination. Twenty litters per herd (10 litters per piglet age category) were identified and 3 piglets within each litter were sampled. Therefore, 60 samples were collected per site. Additionally, herds/sow information along with PCV2 disease and vaccination status were collected from each herd. Serum was tested for PCV2- specific antibodies using SERELISA® PCV2 Ab Mono Blocking ELISA as per the manufacturer's directions. The lower the corrected Sample to Negative ratio (S/Nc), the higher the PCV2 antibody level. The threshold value was 0.5 S/Nc (S/Nc $\geq$0.5 = negative). Serology data were transformed with an appropriate log transformation and summarized by treatment group (sow vaccination status), sow/gilt vaccine type, sow parity and sow/herd PCVAD status.

Results

No Never-vaccinated herds were identified. Multiple vaccination strategies were observed; more vaccination strategies likely exist and are not captured here. Three-days of age piglets were PCV2-antibody positive regardless of maternal vaccination status. Only 21-days old pigs from the During gilt development and As piglet groups were PCV2 antibody negative (geometric mean). The level of MDA by parity was also summarized.

Conclusions and Discussion

Both the timing of sow vaccination and piglet age at sampling appears to influence MDA level. The closer to farrowing sow vaccination was performed, the higher the MDA level in piglets. Three-days of age pigs had numerically more PCV2-MDA than 21-days of age pigs and this is expected as MDA are transferred in colostrum and wane over time. A clear effect of Parity on MDA status could not be determined. It should be noted that MDA may have included antibodies induced via sow vaccination and/or natural exposure.

Acknowledgments

Thank you to the farmers that provided the access to their pigs.

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Porcine Circovirus Type 2 (PCV2) Herd Serology Survey in China

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Introduction

PCV2-associated disease (PCVAD) has become one of the most economically important emerging diseases worldwide. PCV2 vaccination represents a success story to the pig industry globally as most pigs are vaccinated against PCV2. An outcome of the vaccination is the potential presence of maternal derived antibodies (MDA). The objective of the study was to evaluate Porcine Circovirus Type 2 serology in young pigs, specifically maternal derived antibodies (MDA) among and within swine herds in China.

Materials and Methods

Pigs (n=1200) were sampled from 20 herds. Herds were selected based on the PCV2 vaccination status of gilts/sows: During pregnancy (sows were vaccinated for PCV2 at least once during gestation of their current litter and may have been vaccinated previously); During gilt development (sows were vaccinated for PCV2 during gilt development and may have been vaccinated previously); As piglet (sows were vaccinated for PCV2 as a piglet around the time of weaning or before); and Never vaccinated (sows have never been vaccinated for PCV2). Within each herd, two age categories of piglets were sampled: 3 ± 1 days of age and 21 ± 3 days of age; all piglets were sampled prior to piglet vaccination. Twenty litters per herd (10 litters per piglet age category) were identified and 3 piglets within each litter were sampled. Therefore, 60 samples were collected per site. Additionally, herds/sow information along with PCV2 disease and vaccination status were collected from each herd. Serum was tested for PCV2- specific antibodies using SERELISA® PCV2 Ab Mono Blocking ELISA as per the manufacture's direction. The lower the corrected Sample to Negative ratio (S/Nc), the higher the PCV2 antibody level. The threshold value was 0.5 S/Nc (S/Nc ≥0.5 = negative). Serology data were transformed with an appropriate log transformation and summarized by treatment group (sow vaccination status), sow/gilt vaccine type, sow parity and sow/herd PCVAD status.

Results

Multiple vaccination strategies were observed, more vaccination strategies likely exist and are not captured here. Three-days of age piglets were PCV2-antibody positive regardless of maternal vaccination. Only 21-days old pigs from the Never vaccinated group was PCV2 antibody negative (geometric mean). The level of MDA by parity was also summarized.

Conclusions and Discussion

Both the timing of sow vaccination and piglet age at sampling appears to influence MDA level. The closer to farrowing sow vaccination was performed, the higher the MDA level in piglets. Three-days of age pigs had numerically more PCV2-MDA than 21-days of age pigs. A clear effect of Parity on MDA status could not be determined. It should be noted that MDA may have included antibodies induced via sow vaccination and/or natural exposure.

Acknowledgments

Thank you to the farmers that provided the access to their pigs.

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4. Data on File. Zoetis Study Report BH25R-XG-16-658

A North American Serological Survey of PCV2 Antibodies in Pre-Weaned, Non-Vaccinated Piglets

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Introduction

PCV2 vaccines represent the largest segment of the global swine vaccine market. Vaccination of young pigs is very common and use of PCV2 vaccines in sows appears to be growing. Vaccination or natural PCV2 exposure in sows generates PCV2-specific antibodies which can passively transfer to piglets in colostrum. The goal of this survey is to understand how vaccination of sows against PCV2 impacts the presence of maternally derived PCV2 antibody titers in piglets.

Materials and Methods

A total of 19 herds were selected across the U.S. (9 herds) and Canada (10 herds) based on the sow herd's PCV2 vaccination history. Sow farms were classified as follows:

1) Vaccinated During Gestation: Sows were vaccinated for PCV2 at least one time during gestation of their current litter. Sows may have been vaccinated for PCV2 previously. The number of doses given during gestation, and previous vaccination history should be documented.

2) Vaccinated During Gilt Development: Sows were vaccinated for PCV2 during gilt development, prior to entering the sow herd and prior to breeding. Sows were not vaccinated for PCV2 after their first breeding. Sows may have been vaccinated for PCV2 prior to gilt development.

3) Vaccinated As a Piglet (peri-weaning): Sows were vaccinated for PCV2 as a piglet around the time of weaning (as early as one day of age). Sows were not vaccinated for PCV2 after gilt selection.

4) Never Vaccinated: Sows were never been vaccinated for PCV2 during their lifetime.

Within each herd piglets of two age groups (3 ± 1 days of age and 21 ± 3 days of age) were sampled. Ten litters per age group per herd were identified and three piglets from each of these litters were sampled. Litters were also selected based on parity. Healthy piglets were selected for sampling based on size so that a large, medium, and small piglet were sampled from each litter. All sera were tested in the SERELISA PCV2 Ab Mono Blocking kit according to manufacturer's directions.

Results

The number of herds sampled per Sow Vaccination Category is summarized in Table 1.

A single, never vaccinated herd was identified in Canada and no never vaccinated herds were identified in the US. Three-day of age piglets were PCV2 antibody positive if sows had ever been vaccinated. Three day of age piglets had numerically higher PCV2 MDA than piglets at 21 days of age from sows of the same vaccination status. Piglets were negative for MDA at both three and 21 days of age if sows were never vaccinated.

Table 1. Summary of Herds Surveyed by Sow Vaccination Category and Country.

Sow Vaccination Category	US Herds	Canada Herds	Total
During Gestation	3	3	6
During Gilt Development	3	3	6
As A Piglet	3	3	6
Never	0	1	1
TOTAL	9	10	19

Conclusions and Discussion

Piglet MDA trends based on sow vaccination in Canada and the US were biologically similar. Both the timing of sow vaccination and the age of piglet at sampling influence MDA levels. The closer to farrowing sow vaccination is performed the higher the MDA in the piglets. The higher incidence of

MDAs in three day of age piglets is expected as MDA are transferred in colostrum and wane over time. MDAs may or may not include antibodies induced via natural PCV2 exposure. Parity was not shown to clearly affect MDA levels. There are likely additional vaccinations strategies that were not captured in this study.

Characterization of the local and systemic immune response to *Lawsonia intracellularis* infection in vaccinated animals

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Introduction

Porcine proliferative enteropathy, caused by the bacterium *Lawsonia intracellularis*, is well-recognized as one of the enteric diseases of major concern in the swine industry, with a prevalence that can easily reach more than 80% worldwide. Control strategies include the use of a modified live oral and, more recently, a killed injectable vaccine. The purpose of this study was to characterize the local and systemic immune response to *L. intracellularis* infection after oral and injectable vaccination.

Materials and methods

A total of 90 crossbred 3-week-old animals were oral or intramuscular vaccinated, after 21 days pigs received a challenge via intragastric gavage with a 10^8 dose of *L. intracellularis* homogenate. The treatment groups were assigned as follows: 1) Oral vaccinated n=24; 2) Intramuscular vaccinated n=24; 3) Non-vaccinated non-challenged n= 24; and 4) Non-vaccinated challenged n=18. On study days 0, 7, 21 and 28 post infection, six pigs per group were humanely euthanized for sample collection. Sera and intestinal lavages were collected to test for IgG and IgA respectively, to evaluate the humoral immune response and secreted local antibodies, using the immunoperoxidase monolayer assay. Interferon-γ producing cells purified from the peripheral blood mononuclear cells (PBMC), ileum tissue and mesenteric lymph nodes were detected using the ELISPOT (R&D Systems) assay that detects the secretion of IFN-γ by activated or memory T cells.

Results

An animal from the oral vaccine group showed the earliest local IgA secretion, at 7 days after challenge. Twenty-one days after challenge, all pigs showed *L. intracellularis*-specific IgA secretion in both vaccinated groups, with a higher titer for pigs given the oral vaccine.

The systemic humoral IgG response was significantly higher in the intramuscular vaccine group, where 79% seroconverted 21 days post-vaccination. Three weeks after challenge, serum IgG was detected in 100% of the pigs vaccinated with the intramuscular vaccine and 90% of the pigs vaccinated with the oral vaccine.

Pigs from both vaccinated groups showed similar numbers of IFN-y producing lymphocytes from the PBMC and intraepithelial lymphocytes, with no statistical differences among treatments. Prior to challenge, on study day 0, the oral vaccine group had 83% of pigs respond with an average of 16.5 IFN-y producing T cells in mesenteric lymph nodes, while the intramuscular vaccine group had 33% of pigs respond with an average of 2.5 INF-y producing T cells in the mesenteric lymph nodes.

Conclusions

Under the conditions of this study, both vaccines induced local and humoral immune responses after challenge, although with different dynamics. The intramuscularly vaccinated pigs had stronger humoral systemic IgG immune response. An earlier local IgA response was detected in the orally-vaccinated group, with more animals demonstrating a greater number of IFN-y producing T cells in mesenteric lymph nodes after vaccination and prior to challenge.

Comparison of two one dose *M. hyopneumoniae* vaccines in a field trial

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Introduction

Mycoplasma hyopneumoniae (M. hyo) is a bacterial pathogen commonly present in herds all around the world, which affects mainly grow-finishing pigs[1]. M. hyo causes economic losses and it can be worst if other pathogens are involved resulting in the porcine respiratory disease complex (PRDC). In this context, enzootic pneumonia (EP) is a chronic respiratory disease caused by M. hyo that affects the swine respiratory tract, predisposing the lungs to secondary bacterial infections[2]. For this reason, it is important to prevent and control M. hyo in pig farms. Vaccination is a relevant tool to improve performance, decreasing the infection level and reducing clinical symptoms[3]. Therefore, the objective of the present study was to compare piglets' performance from weaning to slaughter with two different vaccines for *Mycoplasma hyopneumoniae*.

Materials and Methods

For this trial, a sow herd was selected at Minas Gerais state in Brazil. Piglets of one farrowing group (n=212, at 21 days of age) were individually identified, weighed and randomly divided into two groups (Group 1=G1 and Group 2=G2). Both groups had equal weights, same number of animals (n=106 by group) and same number of males and females. Piglets were then vaccinated intramuscularly for M. hyo: G1 with Ingelvac MycoFLEX® (Boehringer Ingelheim) (1 ml) and G2 with Hyogen® (CEVA) (2ml). After vaccination, they were randomly distributed in the pens at the same barn under the same management and housing conditions. All piglets were weighed individually again at 56 and 140 days of age. Mean group weights were analyzed using the t-test.

Results

The pigs' performance in this trial is illustrated in Figure 1, which shows mean weights of groups. Mean weight gain of G1 was numerically higher than G2 at 56 (312 g, p=0,42) and 140 (1,59 kg, p=0,26) days of age.

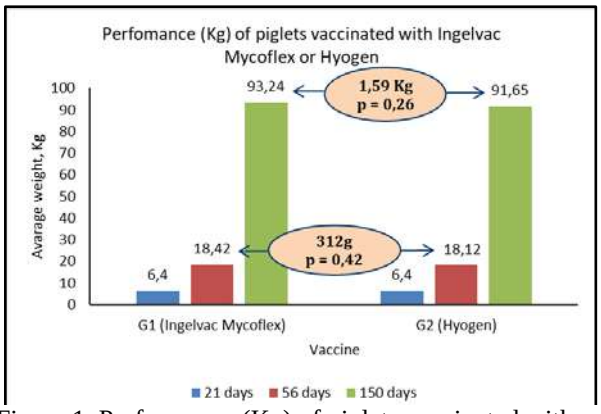

Figure 1. Performance (Kg) of piglets vaccinated with Ingelvac MycoFLEX® or Hyogen®.

Conclusions and Discussion

This study demonstrated a higher main weight gain in pigs vaccinated with Ingelvac Mycoflex® (Boehringer Ingelheim).

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Impact of the use of PCV2 and *Mycoplasma hyopneumoniae* vaccine (reactive and non-reactive) on piglet's performance from weaning to slaughter in Brazil

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Introduction

There are safe and effective vaccines to control porcine circovirus type 2 (PCV2) and enzootic pneumonia with different technologies and adjuvants in the market. Some vaccines, especially those composed by oily adjuvants can cause more severe adverse reactions that can directly affect the daily weight gain[1]. However, there are also vaccines with non-oily adjuvants with high immunogenic potential that do not cause adverse reactions, thus allow a better animal welfare. The aim of this study was to measure the impact of adverse reactions in performance using different vaccines for PCV2 and *Mycoplasma hyopneumoniae* (Mh) in piglets from weaning to slaughter.

Materials and Methods

This study was carried out in a large cooperative in Paraná State, Brazil. 400 male piglets were divided in two groups: T1 (n=200) vaccinated with Ingelvac Circoflex® + Ingelvac Mycoflex® (Boehringer Ingelheim Vetmedica INC) one dose, 2ml (3wk of age) and T2 (n=200) vaccinated with Circumvent PCVM® (MSD Animal Health) two doses, 2ml + 2ml (3wk and 6wk of age). At 21 days old (weaning=D0), each animal were individually identified with a different ear tag. Then, it were randomized by weight and vaccinated according to each group. The piglets were also weighed individually in the end of nursery phase (D43) and before slaughter (D150). The data were submitted to t-test ($p<0.05$).

Results and Discussion

Clinical cases and mortality by PCV2 were not observed in both groups. The performance indexes of each treatment are show in **Table 1**. Piglets from T1 was 0.5 kg heavier than T2 ($p<0.05$) at D43. In growing to finish phase the T1 group kept it higher performance in the final weight (difference between T1 and T2: 2.2Kg, $p<0.05$) and no compensatory gain in animals from T2 group was observed. The worst result in T2 performance was probably due to adverse reaction caused by the oil-based vaccine, mainly after the second dose. It does not happen in T1 animals because of the use of a vaccine with a polymer as the adjuvant that does not cause adverse reactions. Consequently the feed intake and average daily weight gain are not affected according to previous studies[2,3].

Table 1. Body weight (Kg) and average daily weight gain (ADWG) in grams per day in piglets submitted to different vaccination protocols to PCV2 and Mh.

Zootechnical indicators	Treatments	
	T1: Ingelvac Circoflex®+ Ingelvac Mycoflex® non-reactive one dose	T2: Circumvent PCVM® reactive two dose
Weaning weight (D0)	6.51	6.51
Nursery weight (D43)	20.00[a]	19.50[b]
Slaughter weight (D150)	130.70[a]	128.5[b]
ADWG (nursery phase)	313	302
ADWG (finishing phase)	1.035	1.018
ADWG (nursery and finising)	828[a]	813[b]

ab Means with different superscripts within a row differ significantly (Tukey HSD, $P \leq 0.05$).

Conclusions

The choice of a vaccine as a strategy to control swine PCV2 and enzootic pneumonia must take into account effectiveness, practicality and safety, in addition to the impact of adverse reactions on zootechnical performance and animal welfare.

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Zootechnical and economic impact of the use of a live modified oral vaccine (Enterisol®Ileitis) against subclinical *Lawsonia intracellularis* infection in a large cooperative in Brazil

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Introduction

Lawsonia intracellularis (LI) is a swine enteric pathogen that can cause acute or chronic ileitis disease[1]. There is also the subclinical form that is responsible for important zootechnical and economic losses. Vaccination is an important tool to reduce and prevent the LI infection[2]. The aim of this study was to evaluate the effectiveness of vaccination against (LI) with Enterisol®Ileitis through zootechnical and economic indexes.

Materials and Methods

This study was carried out on a large swine cooperative located in the south of Brazil with low occurrence history of porcine intestinal adenomatosis. Ninety-two finishing batches (59,059 piglets) were divided in two groups: T1 – batches with 100% of the animals vaccinated with a live modified oral vaccine against ileitis (Enterisol®Ileitis - Boehringer Ingelheim Vetmedica INC) and T2: batches with unvaccinated animals for ileitis. The piglets of T1 were vaccinated right after weaning (21 days of life in average) with 2 mL orally using a drencher. The animals does not receive any antimicrobial drugs three days before and three days after the vaccination, excepted of Colistin Sulfate present in the feed (dose: 5 mg/Kg). All animals of this study were slaughtered over the same period (between April and December 2016) in order to avoid the effect of seasonality of LI infection. The average daily weight gain (ADWG), feed conversion (FC) and mortality rate were evaluated after the end of each batch and the data were analyzed by Student t-test ($p<0.10$). To calculate the return on investment, specific software (BECAL®) was used.

Results

There was a significant statistic difference in feed conversion ($p<0.10$) between the treatments. The T1 (vaccinated batches) showed an improvement of 43 grams in carcass FC ($p<0.10$) and 7 grams more in ADWG when compared to T2 (non-vaccinated batches) (**Table 1**). There was no statistical difference in the mortality of the two treatments. The return on investment (ROI) was 1:1.43 per animal slaughtered.

Conclusions and Discussion

This result in mortality was expected since the system in question had the subclinical form of the disease. It is important to note that there was no change in the preventive medication protocol in feed between the two treatments. Thus, reduce the antibiotics as preventive to control *Lawsonia intracellularis* is an opportunity to increase the ROI. It was prove that Enerisol®Ileitis has a significant impact in reducing the bacteria shedding by the animals[3].

Table 1. Results of zootechnical indexes comparing vaccinated batches (Enterisol®Ileitis) with non-vaccinated batches.

Zootechnical indicators	Treatments	
	Vaccinated	Unvaccinated
Number of bacths	44	45
Number os animals	32.502	26.557
Inicial weight	23.33	23.46
Final weight	127.02	127.07
ADWG (Kg)	0.834	0.827
Carcass FC (Kg)	3.372[a]	3.415[b]
Mortality (%)	1.85	1.83
Allotment days	124	125

ab Means with different superscripts within a row differ significantly ($P\leq0.10$).

Enterisol®Ileitis proved to be an excellent tool to improve zootechnical indexes with a positive ROI in systems with subclinical ileitis.

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Effect of vaccination with Enterisol® Ileitis on antibiotic consumption and production performance in fattening pigs

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Introduction

Lawsonia intracellularis (Li) is one of the most common enteric pathogens in pigs worldwide[1]. It is the causative agent of porcine proliferative enteropathy (PPE; ileitis), which can affect pigs in different stages of production. Growing pigs most often are affected by the chronic (porcine intestinal adenomatosis or PIA) or subclinical form of the disease[1]. PPE may be treated/controlled using antibiotics or be prevented by vaccination. The objective of this study was to assess the impact of vaccination with Enterisol® Ileitis on antibiotic consumption and production performance in a large fattening farm.

Materials and Methods

The study was conducted in a fattening farm with over 11,500 fattening places, receiving pigs at a mean weight of 26 kg from a single source. Pigs delivered to the fattening site during the observation period either received no vaccine against Li (NON-VAC) or received Enterisol® Ileitis orally via the automatic watering systems equipped with a proportioner at 4-5 weeks post weaning (VAC). Management practices, health status and other vaccinations did not change during the observation period. The average consumption of antibiotics calculated according do Danish regulations created by VetStat (average daily dose per 100 animals per day; ADD100), average daily weight gain (ADWG; g/day), mortality rate (%) and feed conversion ratio (FCR) were compared over a period of 7 months without and 26 months with vaccination against Li. One month in between was excluded as transition period. The data was analyzed by means of statistical process control using Minitab 18.0. Differences in mean values before and after the implementation of vaccination with Enterisol® Ileitis were considered significant with $p \leq 0.05$.

Results and Discussion

The average antibiotic consumption dropped significantly (NON-VAC: 7.97 vs. VAC: 4.45; p=0.028) with vaccination against Li (figure 1).

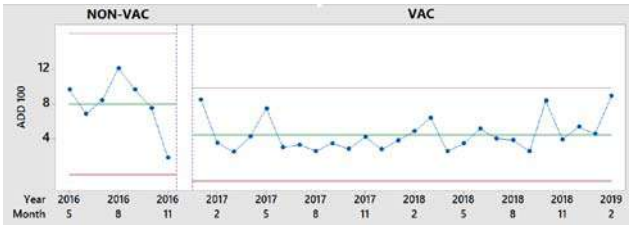

Figure 1: I-MR chart of antibiotic consumption.

The same effect has been shown in previous trials after implementation of vaccination with Enterisol® Ileitis[2,3]. ADWG increased by over 30 g/day, however, differences were not significant (NON-VAC: 935.1 g/day vs. VAC: 965.9 g/day; p=0.368) due to high month-to-month variation (figure 2).

Figure 2: I-MR chart of average daily weight gain.

Mortality rate increased significantly (p=0.02) with vaccination, with a difference of 0.7 % (NON-VAC: 2.16 vs. VAC: 2.87). The suspected cause of death should further be investigated to evaluate the relevance of this change with regards to vaccination against Li. One possible reason could be that other diseases than ileitis contribute to an increased mortality that were previously controlled by broad-spectra antibiotics[4]. No relevant change in FCR could be measured (NON-VAC: 2.683 vs. VAC: 2.679; p=0.979).

Conclusions

In the present study, vaccination against Li significantly decreased antibiotic consumption and numerically improved ADWG. The results further contribute to the body of evidence, that vaccination with Enterisol® Ileitis is an effective tool to reduce the use of antibiotics in pig production.

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Comparison of different swine IgG measuring techniques

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Introduction

Evaluation of colostrum management is possible by measuring maternal derived antibodies (MDA) in new born piglets. Radial immune diffusion (RID) is considered as the gold standard (1), but also expensive for veterinarians in the field, and hardly evaluated for swine. This study compared different techniques on the same set of serum samples for measuring MDA.

Materials and methods

Serum samples obtained from newborn piglets (n= 36, 6 litters with 6 piglets, age 24h) submitted for routine analysis of MDA were used for this comparison study. These 36 samples were combined with 4 negative control sera, collected from still born piglets, resulting in a total of 40 serum samples. Serum samples were all measured with the several available techniques: RID (triple J Farms), Gel Electrophoresis (GE), Zinc turbidity (ZT), Elisa (El. Bethyl swine IgG), immmunocrit (1) (ICa and ICb, 2 laboratories), and for BRIX% using a simple refractometer (RF, Sper scientific).

Results

All four negative control samples appeared negative in the RID assay (not any precipitation visible). The range of IgG concentration in the 36 serum samples was normally distributed and had a mean of 28.2 mg/ml (min 9.46; max 39.3). RID and GE were high correlated (R^2 =96.4%) as is shown in Figure 1. When RID is considered as the gold standard, the other methods R^2 were correlated as follows (in order of correlation): ICa 94.0% (Figure 2); RF 86.8%; ICb 81.1%; ZT 80.0%; El 57.0%.

Discussion

Lab ICb had a lower correlation with RID when compared to ICa. ICa and ICb had a high correlation (R^2=92,8%). The fact that ICb had a lower correlation with RID was probably due to the fact that the used centrifuge had a lower rpm when compared to the centrifuge of lab A resulting in lower centrifugal forces (RCF) (13,000 rpm 16,060 RCF Lab a vs 12,000 rpm 14,825 RCF lab B). This is confirmed by a larger amount of sedimentation of ICb compared to ICa (mean 7.6 vs 6.0 mm). ZT was relative inaccurate in the low range of IgG concentrations (< 15 mg/ml). Elisa has considerable higher variation and contained outliers exceeding the IgG concentrations that were determined by the other techniques.

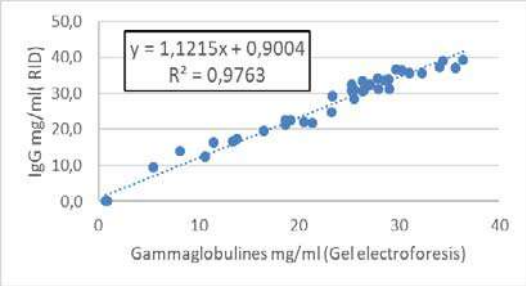

Figure 1 RID (IgG) compared with gel electroforesis (gamma globulines)

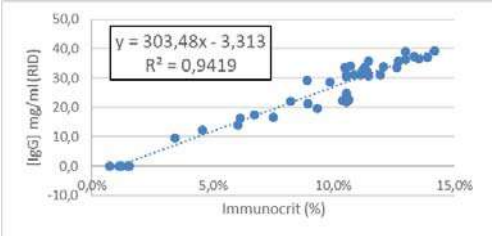

Figure 2 RID (IgG) compared with immunocrit assay (lab a; 15,000 G, % immunocrit ratio)

Conclusion

RID and Gel electrophoresis are well correlated to each other and either one can be used for validation purposes. The immunocrit assay, is relatively cheap , can be performed under field conditions and has accurate results but needs to be validated by each laboratory since differences in centrifugation forces can exist. In conditions of low available (technical) resources, the use of a refractometer (BRIX%) is a simple but quite accurate way of measuring MDA in newborn piglets.

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PRRSFLEX increases average daily weight gain in Dutch finishing pigs

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Introduction

PRRS is circulating in about 85% of the Dutch finishing pigs resulting in an estimated loss of 60 grams in ADG during the finishing pig period (1). In a 600 sow head farrow to finish farm with a historical growth of 783 grams/day, Circo-vaccination was implemented. A group of pigs was also vaccinated against PRRS to evaluate its effect on ADG.

Materials and methods

Growth of in total 1028 was followed during finishing. Pigs were all vaccinated with Ingelvac CircoFLEX® (CF) at 3 weeks of age. In total 339 pigs were vaccinated with Ingelvac PRRSFLEX EU® 2 weeks after weaning at the age of 6 weeks (CPF). Grower pigs were weighed at allocation (18 pig/pen) and again after 15 weeks of finishing. Each pen was scored every week for the presence of conjunctivitis, sneezing, and coughing (0-4; 0 = no signs; 4 = most severe). In total 16 pens were selected for fixed spatial sampling of faces pen swabs (FS) and oral fluids (OF) at 4, 8 and 12 weeks of finishing. FS were tested for the presence of *Lawsonia intracellularis* (Law), OF was tests by PCR for PRRS, Mhyo, SIV and PCV2. At 22 weeks of age 1 blood sample per pen was tested for the presence of antibodies against PRRS, Mhyo, SIV and PCV2. Differences in ADG were tested using Generalized linear model using treatment and sex (SAS).

Results

Oral fluids, faces and serum antibody testing results are presented in Table 1. CPF pigs had higher PRRS SP-ratios when compared to CF (1.87 vs 1.80; P<001).

Table 1 Oral Fluids, feces and antibody testing results.

	Oral fluids samples (PCR)				**AB Elisa**
Age (w)	**6**	**10**	**14**	**18**	**22**
PRRS	6%	75%	13%	0%	100%
PCV2	19%	69%	46%	47%	32%
SIV	56%	6%	20%	27%	51%
Mhyo	0%	0%	0%	0%	14%
	Faces pen swabs				
Law		0%	25%	17%	82%

ADG results are presented in Table 2. ADG of CPF pigs were higher when compared to CF pigs (937 vs. 894 gram/day; p<0.05). ADG of boars was higher when compared to gilts (p<0.05).

Table 2 ADG of the finishing pigs (gram/day). CPF pigs had a significant higher ADG (P<0,05) when compared to CF.

Treatment	Sex	ADG	ADG
CF	Boars	915	894
CF	Gilts	872	
CPF	Boars	955	937
CPF	Gilts	918	

CF pigs had higher clinical score for eyes (0.24 vs 0.10) and sneezing (0.14 vs 0.11). Differences of the eye score were most prominent and presented in Figure 1. Only minor symptoms like black eye lids (score 1) were observed which faded away during finishing. CPF pigs had a significant lower eye score during the first 6 weeks (P<0.001).

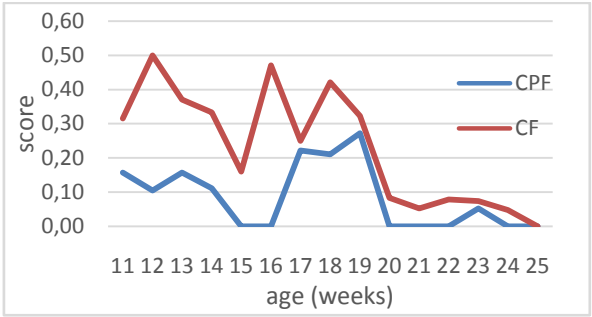

Figure 1 Results from weekly scoring of the eyes during the growth-finishing phase.

Discussion

There was an increase of 43 grams in ADG in the CPF group. With a gross profit of €30.51 per pig (Dutch 2018 average) (2) this results in 30.51/894=€0.034 extra benefit per gram growth increase. The improvement of 43 grams result in an economic benefit of 43*€0.034 = €1.46 per pig. It was not possible to measure FCR, but assuming that FCR will improve when ADG is improved, the probable result will even be (far) larger.[

Conclusion

PRRSFLEX increased ADG with 43 grams (4,8%) resulting in an improved economical benefit, after deduction of vaccine costs.

References

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2 Dutch gross finishing pig profit 2018.

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Acute phase response after intramuscular ileitis vaccination reduces feed intake when compared to both a control group and an oral vaccinated group

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Introduction

Vaccinations via the intramuscular (IM) route are widely used in the pig industry. An oral live vaccine has been available for control of ileitis globally. Recently, a killed oil-adjuvanted intramuscular vaccine for the control of Ileitis has been registered in the EU. The acute phase proteins Haptoglobin (HG) and Pig-MAP (PM) are sensitive indicators for health and welfare of pigs (1). The objective of this study is to compare the available Ileitis vaccines with a non-vaccinated control group on the inflammatory status by measuring acute phase response and the feed intake the first week after vaccination.

Materials and Methods

On a commercial Dutch finishing pig farm using a liquid feeding system, a weekly batch of 900 pigs was selected. Pigs arrived at the age of 10 weeks and weighed 24.1 kg at the start of the trial. Pigs were divided at random in 3 groups: a non-vaccinated control group (CON), an oral vaccinated group (Enterisol® Ileitis and an intramuscular vaccinated group (Porcilis® Lawsonia, MSD (PL)). Vaccine was administered by the local veterinarian according to the manufacturer's instruction at one day after allocation in the pig barn. The day after vaccination, per group 15 serum blood samples were taken for the determination of the acute phase proteins HG and PM (Dutch Animal Health Service; GD Deventer). Daily feed intake was recorded by the liquid feeding machine. Statistical analysis was carried out using Minitab for the acute phase proteins (non parametric Mann Whitney test) and excel for the cumulative feed intake 7 days after vaccination (two sided t-test).

Results

The intramuscular injection with PL evoked a significant ($P<0.001$) increase in both of the tested acute phase proteins HG (2.02 mg/ml) and PM (2.28 mg/ml) when compared to CON (HG 0.70 mg/ml; PM 0.50 mg/ml) and ENT (HG 0.61 mg/ml; PM 0.47 mg/ml). CON and ENT did not differ (Figure 1).

The cumulative feed intake was significantly ($P<0.001$) lower for the PL group (13.2 kg) when compared to CON (14.0 kg) and ENT (14.0 kg) (Figure 2). Feed intake differed as well for the cumulative feed intake at day 1-3 (CON 4.0 kg; ENT 4.0 kg; PL 3.1 kg; $p<0.001$) and the feed intake at day 2 (CON 1.4 kg; ENT 1.4 kg; PL 0.7 kg; $P<0.001$). Based on an feed conversion ratio of 1.77 in early finishing (2) the reduction of 0.8 kg feed intake in seven days' time equals 0.45 kg growth reduction during the 7 days of the trial.

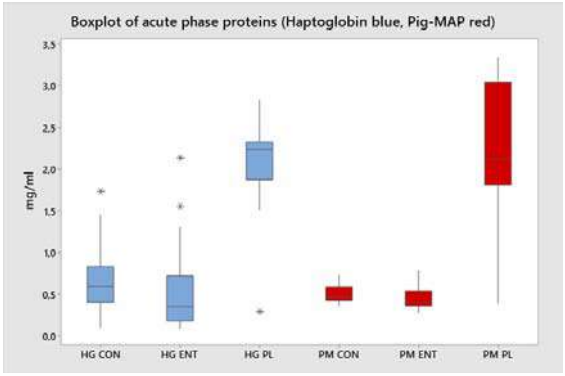

Figure 1. Boxplot of the acute phase proteins Haptoglobin (HG) and Pig-MAP (PM) for the unvaccinated control (CON) oral live (ENT) and IM (PL) groups. PL had a significant ($P<0.001$) higher concentration of both HG and PM compared to CON and ENT. CON and ENT did not differ from each other.

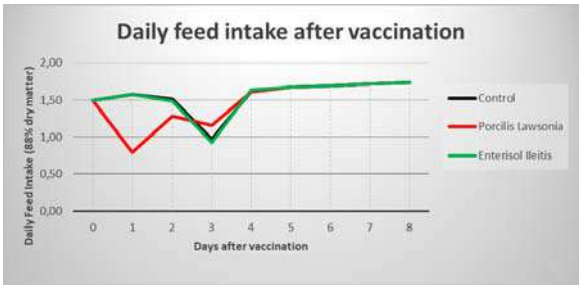

Figure 2. Daily feed intake dropped after PL vaccination. The feed intake at day 2 after vaccination and the cumulative feed intake the first week after vaccination was significant lower (0.8 kg per pig) for the PL group ($P<0.001$).The drop at day 4 for CON & ENT was a correction of the high feeding level.

Conclusions and Discussion

Oral live vaccination has been proven efficacious, also compared to an intramuscular vaccination (3). The use of an oral live vaccine did not evoke a systemic inflammatory acute phase response in line with other studies (4) and had no negative effect on feed intake when compared to the control group. The intramuscular injection led to an increase of inflammation as measured by acute phase proteins. This was followed by a lower feed intake the first week of finishing.

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Non-specific effects of rabies vaccine on mortality and antibiotics consumption in a Danish pig herd

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Introduction

Vaccines may have non-specific effects, decreasing or increasing susceptibility to other infections than the targeted disease by enhancing or weakening the immune system. Non-specific effects have been observed in many human vaccines, including the tuberculosis vaccine BCG (live bacterial), oral polio vaccine (live viral), measles vaccine (live viral), and diphtheria-tetanus-pertussis vaccine (inactivated) [1]. The general observation is that live vaccines have beneficial non-specific effects, whereas inactivated vaccines may have adverse non-specific effects. Maternal priming with live vaccines seems to amplify the beneficial non-specific effects of the same vaccines in the offspring [2].

In a recent trial of a new malaria vaccine RTS,S, girls receiving the malaria vaccine worryingly had a 2-fold increased all-cause mortality compared to the girls who received an inactivated rabies vaccine, used as a comparator vaccine [3]. The explanations for the adverse association could be a female specific adverse non-specific effect of the malaria vaccine. Alternatively, it was hypothesized that the rabies vaccine had beneficial protective effects on mortality. [4]. A few historical experimental studies in mice and lately also a study in free roaming dogs have indicated that rabies vaccine non-specifically reduces overall mortality [5]. Overall, whereas there is increasing evidence of non-specific effects of human vaccines, there could be potential positive non-specific effects of veterinary routine vaccines, which could increase the overall survival rate for piglets and young pigs. The effect could especially be beneficial for various infectious diseases for which there is no other preventive alternatives e.g. viral and bacterial diseases where no, or inadequate, vaccines are available. The present study was designed to investigate if the veterinary inactivated rabies vaccine may have non-specific effects on morbidity and mortality in young pigs. In addition, the potential interaction with maternal vaccination was explored.

Materials and Methods

In a Danish commercial SPF-herd free of PRRS, but with positive SPF status for *M. hyopneumoniae* and *A. pleuropneumoniae serotype* 12, 575 pregnant sows (2-3 weeks before scheduled farrowing) and 5747 of their subsequent offspring (median 6 days of age) were allocated (1:1) to rabies vaccine or no rabies vaccine, using a 2x2 factorial randomized design. Except for the rabies vaccine, all animals were treated as customary for the herd. The herd had a pre-study average preweaning mortality of 16.2% and a mortality from weaning to 30 kgs of 1.2% within the 120 days prior to the study. Deaths and antibiotic treatments were observed until departure from the observation stables (approximately 12 weeks of age /weight 30 kgs). Wildtype rabies are not present in Danish pig herds.

Results

Until 21 days of life, 90 pigs died (overall mortality rate=1.6%). Of these, 50 had received rabies vaccine and 40 were controls, the mortality rate ratio being 1.27 (95% confidence interval: 0.85-1.90). Prior sow rabies vaccination did not modify the effect of neonatal vaccination. For mortality as well as antibiotic treatments within 21 days of life, there was non-significant indication of a beneficial effect of rabies vaccine in female piglets, but a negative effect in (castrated) male piglets from rabies-naïve sows, and a negative effect of rabies vaccine in female piglets from rabies-vaccinated sows. These effects had waned at 12 weeks of age.

Conclusions and Discussion

With a slightly higher mortality in the piglets receiving inactivated rabies vaccine, the study did not support the hypothesized beneficial non-specific effect of rabies vaccine. Although the low mortality impaired the power of subgroup analyses, the study indicated effect modification by sex and maternal vaccination.

This study was performed in a high health herd with a low pig mortality after day 5 (median age of piglets at randomization was 6 days). This could lower the effect of the rabies vaccine, and therefore the results could be different in a low health herd with higher mortality and more infectious related disease problems.

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Validation of a PRRSV live-virus potentiated by replication-competent expression of porcine interferons

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Introduction

Although several PRRSV vaccines are currently available, based on different field isolates, they show limited cross-protective efficacy. In contrast to traditional methods of attenuation of PRRSV through repetitive cell culture passage, interferon genes were cloned into a PRRS DNA-launched reverse genetics system to generate vaccine candidates with specific changes. This recombinant virus was evaluated *in vitro* and *in vivo* to determine the effects of transgene expression on the virus, the cell, and the host animal. This vaccine platform is designed to directly overcome PRRSV-induced suppression of the pig's IFN signaling and associated immune response, in a manner similar to the atypical, IFN-inducing PRRSV strain A2MC2 [1-3]. Thus it may enhance vaccine efficacy against both homologous and heterologous PRRSV strains. Initial studies have shown the vaccine prototype efficacy is comparable to a commercially available PRRSV MLV vaccine.

Materials and Methods

The pCMV-P129 infectious cDNA clone was constructed from the virulent PRRSV-2 (Linage 8) field virus P129, isolated in Indiana in 1995. The infectious clone pKermit (Zoetis) [4] contains the GFP gene within an additional dedicated sgRNA expression cassette. The virus "Kermit" is derived from pKermit and causes infected cells to fluoresce with green light. In this study, the GFP gene was replaced with genes encoding a cohort of optimized antiviral interferons (IFNs) including each of IFN-α, IFN-β and IFN-ω subtypes. The IFN cohort-expressing virus (PRRSV-P129-IFNmix) was tested with or without an adjuvant and compared with a commercial MLV vaccine, Ingelvac PRRS® ATP (MLV-ATP). The vaccine challenge study used outbred pigs (5-wk-old, n = 10/group) (Table 1.)

Table 1: Experimental design for the PRRSV-P129-IFNmix challenge study

Group	N	Vaccine	ToFA (5ug/ml)	Montanide gel 10%	Challenge
1	10	Sham		+	NADC-34
2	10	Sham	+	+	NADC-34
3	10	PRRSV-P129-IFNmix		+	NADC-34
4	10	PRRSV-P129-IFNmix	+	+	NADC-34
5	10	PRRSV-P129-IFNmix			NADC-34
6	10	Sham			NADC-34
7	10	MLV-ATP			NADC-34

PRRSV dose: 2 ml/pig IM at 1 x 10^4 TCID50/ml; Sham: cell culture medium; Adjuvant: 10% Montanide T M IMS, Seppic; MLV-ATP: Ingelvac PRRS® ATP vaccine; ToFA: an acetyl-CoA carboxylase-α inhibitor.

Results

The PRRSV-P129-IFNmix virus had a higher replication rate and generated 0.5-2 logs more vaccine virus than the MLV-ATP vaccine during the 4-wk vaccination period. After the pigs were challenged with NADC-34 strain, all PRRSV-P129-IFNmix treatments were more effective in suppression of the challenged virus regardless of inclusion of the adjuvant (ADJ, 10% Montanide™ IMS, Seppic) [5]. The febrile response in pigs vaccinated with the attenuated PRRSV-P129-IFNmix are similar to the commercial ATP vaccine, particularly post challenge. The PRRSV-P129-IFNmix groups had a statistically non-significant difference across the range of high gross lung lesion scores from the MLV-ATP vaccine. The weekly weight gain in pigs vaccinated with the attenuated PRRSVp129-IFNmix vaccine candidates in comparison to the commercial ATP vaccine. The MLV-ATP group had better weight gain pre-challenge, but the groups with the tested vaccine candidates, particularly the MLV-PRRSVp129-IFNmix-ADJ, had greater weight gain post challenge. At -42 DPI all treatment groups were PRRSV antibody negative and PRRSV-free at vaccination. At challenge (0 DPI), the control and non-vaccinated groups were PRRSV antibody negative, while each vaccinated group was antibody positive. At 14 DPI, all challenged groups had antibody titers that were significantly higher than the non-vaccinated, non-challenged sham group; and the vaccinated groups were significantly different from the non-vaccinated groups.

Acknowledgments

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Study of the correlation between the serology for Swine Erysipelas in the sow and her offspring

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Introduction

Colostrum intake is a critical point in the health management of swine farms, as different studies have directly correlated it to pre-weaning piglet mortality[1].
Colostrum has different roles in piglets; nutritional, as a source of energy for the neonatal piglet[2], physiological, as a source of hormones for improvement of gut and reproductive maturation in the future[3], and immunological, as a source of acquired immunity (humoral and cellular).[4]
Nearly 100 % of sows are vaccinated against Swine Erysipelas (SE) in Europe. Furthermore, SE titers are transferred by colostrum (maternally derived immunity (MDI))[5], and SE titers can be quantified in both sows and piglets using an SE indirect ELISA kit, with robust results. Therefore, a direct correlation between a sow's titer and her offspring's (piglet's titer) might be assumed in the case of good colostrum intake.

The aim of this study was to validate a new method of colostrum intake assessment through SE titers of the sow and her offspring (piglets) at lactation.

Materials and methods

13 farms in France that were routinely vaccinated against SE and Porcine Parvovirus (PPV) during lactation (3 weeks before the next artificial insemination) were selected. On each farm, 10 post-farrowing sows of different parity were selected and blood samples were taken at approximately day 7-10 of lactation, before the next vaccination, as well as 3 medium-sized piglets from each selected sow (30 piglets in total per farm). No cross - fostering was done within the first 48 hours post-farrowing, so that, each piglet took the colostrum from its own mother. Blood samples were analyzed with CIVTEST® SUIS SE/MR (*Erysipelothrix rhusiopathieae* indirect ELISA kit, cut-off: 40) and direct sow-piglet correlation was analyzed by Spearman correlation test.

Results

There was a strong correlation between the *Erysipelothrix rhusiopathieae* antibody titers of the sows and the titers from their respective piglets (R=0.66, *p-value* < 0.001).
The correlation was seen not only in the overall data but also when each farm and each animal was assessed individually (Fig. 2).

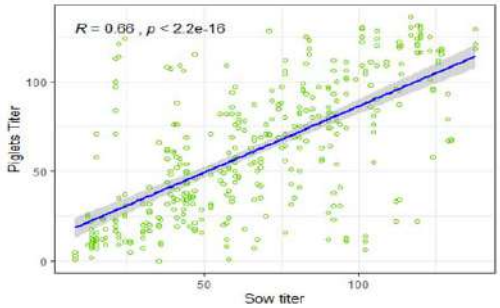

Figure 1. Scatterplot showing the correlation of Erysipelothrix rhusiopathiae antibody titers between sows and piglets.

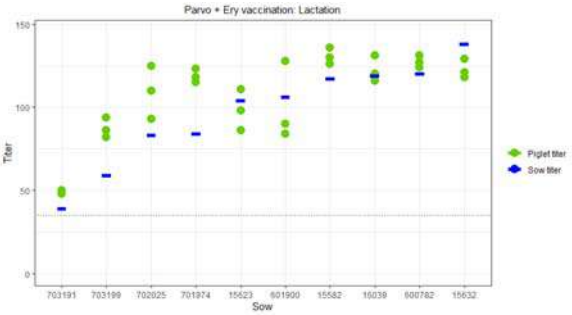

Figure 2. Correlation between sows and their piglets on a farm in France.

Conclusions and discussion

This study confirms the high correlation between a sow's SE titer and her piglet's SE titer when the sampling was done at day 7-10 of lactation. Therefore, in the cases where this correlation was not seen, we might assume poor colostrum intake by the piglet. Consequently, the SE colostrum intake test could be a useful tool for swine practitioners to investigate the quality of colostrum intake and differentiate between farms.
However, further studies should be done to determine the difference in terms of good/medium/poor colostrum intake on different farms and correlate this with pre-weaning mortality and other productive parameters.

Acknowledgments

This survey was carried out on farms under health monitoring by the Triskalia team (Farming monitored in PSE).

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Comparative trial of the humoral immune response against Swine Erysipelas elicited by two different trivalent vaccines

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Introduction

Erysipelothrix rhustiopathiea is a ubiquitous facultative intracellular gram-positive bacterium that is found worldwide[1]. It can be isolated from a variety of environmental samples such as manure, oral fluids, feed, or surfaces such as walls or fans[2]. Antimicrobial therapy could be used in affected pigs to control this disease, however, recent studies have shown an increase in resistance to different antibiotics of different Erysipelas' strains[3]. Therefore, prevention of Swine Erysipelas (SE) is best accomplished by immunization programmes[1].

The aim of this study was to assess and compare the humoral immune response against SE elicited by two reproductive vaccines, over a period of 71 days.

Materials and Methods

A controlled experimental trial was performed in 30 SE-negative animals that were randomly assigned to three groups (n=10). Group 1 (G1) was vaccinated with ERYSENG® PARVO/LEPTO (trivalent vaccine against SE, Porcine Parvovirus (PPV) and *Leptospira sp.*) adjuvanted with HIPRAMUNE® G, Group 2 (G2) with Vaccine B (trivalent vaccine against SE, PPV and *Leptospira sp.*) adjuvanted with dl-α-tocopheryl acetate, and group 3 (G3) was injected with PBS as a control group. All the groups were vaccinated and re-vaccinated intramuscularly following the instructions contained in the SPC.

Serum samples were taken on days -21, 21, 36, 49 and 71 after vaccination. SE serology was performed by using a commercial indirect ELISA kit (CIVTEST® SUIS SE/MR). This ELISA kit's suitability for the detection of anti-SE antibodies without bias towards any of the vaccines was previously reported[4].

Results

All the animals were negative against SE before the administration of the products. In addition, significant differences (Mann-Whitney U test; $p<0.05$) between groups were observed from day 21 to day 71 of the study, which could be directly attributed to the vaccines, as the control group remained negative within the study.

All the animals from G1 and G2 were positive throughout the study, however, the humoral immune response elicited by G1 (ERYSENG® PARVO/LEPTO) was the highest throughout the trial with statistically significant differences (Mann-Whitney U test; $p<0.05$) on days 21, 36, 49 and 71 of the study.

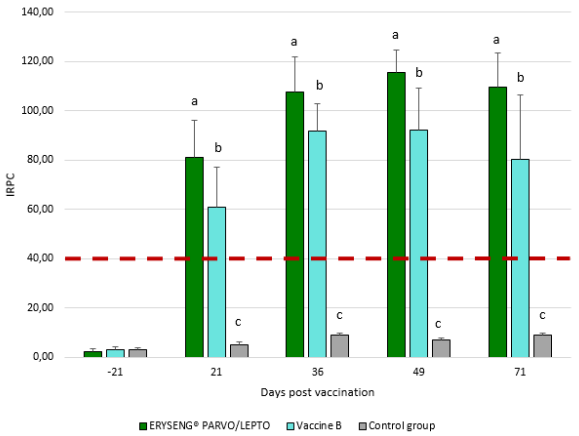

Figure 1. Results are represented as average and standard deviation. Different letters indicate statistically significant differences (Mann-Whitney U test; p<0.05). The slashed red line indicates the cut-off (40 IRPC).

Conclusions and Discussion

Both humoral immunity and cell-mediated immunity play a key role in the host defence against *E. rhusiopathiae* infection. However, cell-mediated immunity and its relative contribution to protection against SE are currently unknown[1]. Therefore, this trial focusses attention on the humoral immune response.

The tested trivalent vaccines were licensed for protection against SE and, as expected, they produced an active and specific antibody immune response in this study. However, ERYSENG® PARVO/LEPTO produced a notably higher immune response compared to the other tested product.

This differential characteristic might be a useful resource in the field, as previous observational studies have demonstrated that seronegative subpopulations of pigs can be found on farms using vaccination against SE[5]. Providing a higher antibody response might make it possible to cope with those situations, by avoiding having clinical and sub-clinical problems related to Swine Erysipelas

Acknowledgments

The authors wish to thanks the UCAM unit of HIPRA for their technical support.

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Comparative trial of the safety of two reproduction vaccines applied on lactation

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Introduction

The optimum vaccination protocol for reproductive vaccines against Swine Erysipelas (SE), Porcine Parvovirus (PPV) and *Leptospira sp.* is during lactation, thus preventing future reproductive problems during the next gestation. The impact that these vaccines could produce to the sow and gilts influences their welfare, feed intake, milk production and consequently piglet performance during lactation.

The aim of this study was to assess and compare the safety, in terms of local reactions and increase of body temperature, of two trivalent vaccines applied during lactation.

Materials and Methods

A controlled and blinded experimental trial was performed with 27 lactation sows. These animals were randomly assigned to three groups; Group 1 (G1, n=10) was vaccinated with ERYSENG® PARVO/LEPTO (trivalent vaccine against SE, PPV and *Leptospira sp.* adjuvanted with HIPRAMUNE® G), Group 2 (G2, n=10) was vaccinated with Vaccine B (trivalent vaccine against SE, PPV and *Leptospira sp.* adjuvanted with α-tocopherol), and Group 3 (G3) was injected with PBS as a control group. All groups were vaccinated approximately at day 16 of lactation.

Rectal temperature (RT) and local reactions (LR), in terms of inflammation at the inoculation point, were evaluated 24 hours before vaccination, at vaccination moment, 6 hours post vaccination (hpv), 24 hpv and 48 hpv.

Results

At vaccination moment, none of the animals had fever (TºC<38.5ºC). However, 6 hpv significant differences were found between Vaccine A and Control group (p<0.05), with more than half of a degree of difference. Furthermore, the increase in rectal temperature of Vaccine A was higher than EPL from 6 hpv to 48 hpv as seen on Table 1.

Table 1. Mean increase in RT of the different groups during the study. ANOVA test was performed to analyses the differences between groups. Different subscripts mean significant differences (p<0.05).

Time	Control	Vaccine A	ERYSENG® PARVO/LEPTO	P-value
Reference	-1 day	-1 day	-1 day	
0	0,345	0,325	0,327	0,99
6 h	$0,288^{A}$	$0,865^{B}$	$0,789^{AB}$	0,04*
1 day	0,168	0,517	0,068	0,15
2 days	0,133	0,07	0,04	0,90

Moreover, there was significant differences in terms of LR between G2 and control group at 6 hpv (p<0.05). No differences were observed between EPL group and the control group at any time point of the study.

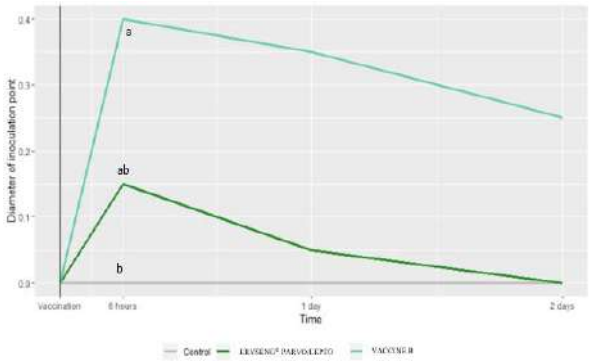

Figure 1. *Mean diameter of the inoculation point of the different groups during the study. Kruskall-Wallis test was performed to analyse the differences between groups. Different subscripts mean significant differences (p<0,05).*

Conclusions and Discussion

This study shows significant differences between both vaccines in terms of safety (temperature increase and local reactions). Sometimes, safety issues could be seen as being of minor relevant in swine production, but this is indeed a crucial aspect, as the sow is at a physiological complex stage (lactation or gestation). An increase of temperature (fever) could involve an abortion[1] or a reduction of feed intake that would lead to lower milk production and consequently, lower piglet performance at weaning[2]. Moreover, the inflammation at the inoculation point could lead to local abscess and future welfare problems.

In conclusion, safety concerns must be taken into consideration when evaluating vaccine performance in sows.

Acknowledgments

HIPRASTAT team and HIPRA PORTUGAL team for their support.

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Assessment of the efficacy of ERYSENG® PARVO/LEPTO in Thailand

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Introduction

Swine Erysipelas (SE), Porcine Parvovirus (PPV) and *Leptospira sp.* are very common organisms of infectious etiology that can cause reproductive diseases in pigs. However, infection with either of these agents mainly produces subclinical disease, but animals may not die in larger or smaller-scale herds[1,2]. Even so, the high incidence of SE has increased since the rapid emergence of resistant bacteria attributed to overuse and misuse of antibiotics[3]. ERYSENG® PARVO/LEPTO **(**EPL) is a triple-action vaccine (SE, PPV and *Leptospira interrogans sp.*) with broad protection against three of the most significant diseases affecting porcine reproductive health. Currently, no published large-scale analysis has assessed the efficacy of EPL in Thailand.

The aim of this study was to evaluate sow performance before and after the implementation of a vaccination schedule using the EPL vaccine.

Materials and Methods

The safety and efficacy of ERYSENG® PARVO/LEPTO **(**EPL) were assessed on a farm of 1,260 healthy sows in the western part of Thailand during 2018-2019. The farm history was considered typical of SMEDI with a high incidence of average mummified fetuses and average fetal deaths of up to 5.46% and 9.45%, respectively.

The farm was using a triple action vaccine (Vaccine A, market leader in Thailand) but experienced severe to moderate side effects after injection, including depression, skin rash, vomiting and respiratory distress. Since the last doses of vaccine A, the sows (n =1,208) had received 2 doses of EPL administered in accordance with the manufacturer's leaflets. The safety of the vaccine in sows was monitored daily for adverse reactions up to 72 hours post vaccination. The field efficacy of the vaccine was determined by comparing sow performance before (Vaccine A**)** and after using the EPL vaccine. Furthermore, reproductive parameters in this trial were statistically analyzed using the SPSS statistical program (version 22.0).

Results

Regarding the safety assessment, no local and systemic reactions associated with EPL vaccination were observed. In terms of efficacy, group mean sow productivity with EPL showed that improvement in reproductive performance was significant, as shown in Table 1 **(**$p<0.05$**)**, specifically with a reduction in mummified fetuses of up to 47.56 %. Furthermore, the average daily gain (ADG) of the piglets during lactation also increased by 17.85 g/day ($p<0.05$) compared to Vaccine A, which showed a 9.76 % increase.

Table 1. Production parameters measured in the subsequent gestation, for a set of each vaccine group (EPL vs Vaccine A).

Production parameter[1]	EPL	Vaccine A	*P-value*	Diff **(%)**
No. of sows	1,260	1,208		
Farrowing rate (%)	92.75[a]	88.18[b]	0.042	+5.18
Total number of piglets born alive /litter	13.9[a]	12.15[b]	0.003	+14.4
Stillbirths (%)	7.9[a]	10.23[a]	0.060	-22.78
Mummified fetuses (%)	3.12[a]	5.95[b]	0.049	-47.56
Pigs weaned/sow	11.7[a]	9.62[a]	0.124	+21.62
Pre-weaning mortality (%)	6.73[a]	16.63[b]	0.0006	-59.53
ADG (g/day)	200.71[a]	182.86[b]	0.0019	+9.76

[1] Sow performance was recorded from 2 groups (EPL vs Vaccine A): different superscripts (a, b) indicate statistically significant differences within the main parameters ($p \leq 0.05$)

Conclusions and Discussion

These results indicate that the EPL vaccine is safe and efficacious in reducing the rate of pre-weaning mortality in piglets, mummified fetuses and stillbirths under field conditions. The ADG was also statistically significantly higher, which could be related to the better safety of EPL in lactation compared to Vaccine A. Furthermore, field data provide key general insights into the evaluation of the impact of the EPL vaccine that should be considered in the control of these diseases in swine herds in Thailand.

Acknowledgments

The authors would like to express their gratitude to the HIPRA THAILAND team and farm owners from Thai pig-farming cooperatives for providing retrospective data and for their support.

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Improvement of productive parameters after using ERYSENG® PARVO/LEPTO on a farm in Colombia

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Introduction

Reproductive problems in sows are multifactorial and can be divided into two large groups: non-infectious problems, which are those produced by genetics, nutrition, management or environmental causes, and infectious problems, which may be caused by viruses or bacteria[1]. In this study, we will focus on infectious problems, and namely those caused by three of the most prevalent pathogens worldwide and which cause the most significant reproductive and economic losses, namely Swine Erysipelas (SE), Porcine Parvovirus (PPV) and *Leptospira sp.*. In any case, there are significant differences between different vaccines available on the market, in relation to humoral immune response and safety[2,3].

The study objective was to compare the productive and reproductive performance before and after implementing a vaccine programme using ERYSENG® PARVO/LEPTO (inactivated vaccine against SE, PPV and *Leptospirosis*) adjuvanted with HIPRAMUNE® G on a Colombian commercial farm.

Materials and Methods

The study was conducted on a 700 sows farrow to finish farm located in the Valle del Cauca (Colombia). The farm was negative for PRRS, Progressive Atrophic Rhinitis, *Haemophilus parasuis* and Flu, and has not had any disease outbreaks during the last few years. The farm had good biosecurity, and there were no changes to staff, management or genetics in the past few years.

In this study, two consecutive time periods were studied (April 2016 to March 2017) and (April 2017 to March 2018), and thus seasonal bias was avoided.

In the first period on the farm, the vaccine A adjuvanted with Amphigen® was used following the vaccination programme recommended by the manufacturer, and in the second period the ERYSENG® PARVO/LEPTO (EPL) vaccine was used. The vaccination programme used with EPL was one vaccination in the whole herd plus re-vaccination three weeks later, and after that two doses before inseminating the nulliparous sows, and one dose in each cycle in the multiparous sows. All productive and reproductive data was collected from both periods and was analysed statistically using the Wilcoxon test.

Results

The total piglets born per sow per farrowing (TPBS) significantly increased from 13.25±0.35 to 14.48±0.27 ($p < 0.001$) and consequently the piglets born alive per sow (PBAS) also increased from 12.38±0.318 to 13.34±0.27 ($p < 0.001$)

Table 1. Productive and reproductive parameters between April 2016–March 2017 (Vaccine A), and April 2017–March 2018 (EPL). Terminology: PWSY: (Piglets weaned per sow per year) TPBS: (Total piglets born per sow) PBAS: (Piglets born alive per sow) SS: (Stillbirths per sow) ML: (Mummified per litter) WPS: (Weaned piglets per sow) %LL7: % of litters with less than 7 piglets born alive.

	Vaccine A	ERYSENG® PARVO/LEPTO	Difference	P-value (Wilcoxon test)
PWSY	28.27 ± 0.72	30.3 ± 0.90	+2.03	0.002
TPBS	13.25 ± 0.35	14.45 ± 0.27	+1.2	< 0.001
PBAS	12.38 ± 0.32	13.34 ± 0.27	+0.96	< 0.001
SS	0.507 ± 0.03	0.63 ± 0.06	+0.12	0.003
ML	0.37 ± 0.05	0.48 ± 0.08	+0.11	0.035
WPS	11.60 ± 0.31	12.59 ± 0.24	+0.99	< 0.001
%LL 7 piglets	7.57 ± 1.77	3.83± 1.53	-3.74	0.004

The fact that the total births and live births increased implies that the mummified and stillborn piglets increased slightly, since the litter size is larger, but ultimately, the piglets weaned per sow per year (PWSY) increased by more than 2 piglets per sow (28.27±0.72 to 30.3±0.90) ($p < 0.05$). Another parameter that was significantly reduced was the percentage of litters with less than 7 piglets born alive (%LL7) which changed from 7.57±1.77 to 3.83±1.53 ($p < 0.05$). This may indicate better control of infectious diseases such as PPV.

Conclusions and Discussion

Following implementation of ERYSENG® PARVO/LEPTO, the productive parameters increased significantly, which entails greater productive and economic profitability for the farm. This study demonstrates the importance of having a good vaccination plan against Swine Erysipelas (SE), Porcine Parvovirus (PPV) and *Leptospira sp.* which produces a robust herd immunity and consequently significant improvement in the farm.

Acknowledgments

The authors wish to thank Jaime Eduardo Escobar González.

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Safety assessment of ERYSENG® PARVO/LEPTO and two commercial reproductive vaccines in Thailand

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Introduction

Triple vaccination plan against Swine Erysipelas (SE), Porcine Parvovirus (PPV), and *Leptospira interrogans sp.* in one shot is widely used in pig farming worldwide[1] including Thailand. ERYSENG® PARVO/LEPTO (EPL) is a new inactivated vaccine against SE, PPV and *Leptospira interrogans sp.* containing a state-of-the-art aqueous adjuvant developed by HIPRA and based on ginsenosides (HIPRAMUNE® G[d]). The safety of bacterial vaccines has always been a concern. Gilts receiving these vaccines for the first time and sows being given during the lactation period could affect health and milk yields[2].

The aim of this study was to compare how the difference in safety between different reproductive vaccines could affect the production performance of sows and piglets up to weaning under field conditions in Thailand.

Materials and Methods

The study was split into two parts, the first one to evaluate the safety of EPL, and the second one to compare the safety of EPL safety with two other vaccines:

EPL Safety test: This study was conducted on twenty commercial Thai pig farms, working in a four-batch system during January to September 2019. EPL was administered by the IM route (2 ml). The EPL vaccination program for gilts was two doses at 6 and 3 weeks prior to artificial insemination. Sows were vaccinated 10 days after farrowing, i.e. during lactation. On the three days after vaccination, the injection site was evaluated in gilts and sows, scoring the size of skin swelling from 0 to >3 mm. Behavioral performance was also monitored daily after vaccination.

Safety comparison between EPL, vaccine A and vaccine B: Two pig farms were selected to compare the field safety of EPL and two other commercial trivalent vaccines. Vaccine A (market leader in Thailand), and Vaccine B (old vaccine), were evaluated. Some parameters were compared between groups using a statistical program (IBM SPSS statistics 22 program).

Results:

EPL Safety test: A total of 136,800 vaccine doses were administered on 20 pig farms and 11 vaccine-associated mild side effects were reported (0.00008%). Only 7 of the 11 adverse events were vaccine-related in gilts. These vaccinated animals returned to normality two days post vaccination without any medical treatment. Adverse reactions in gilts and sows are shown in Figure 1.

Safety comparison between EPL, vaccine A and B: EPL-vaccinated sows and gilts had significantly (*p-value*<0.05) increased feed intake and significantly lower adverse reactions compared to the other two commercial vaccines as shown in Table 1.

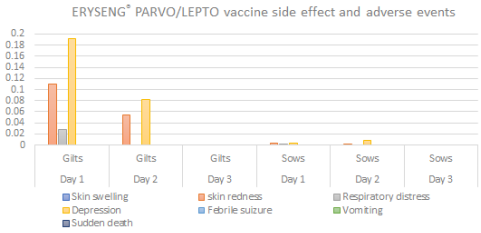

Figure 1. Local injection site and systemic reactions in gilts and sows after vaccination with EPL

Table 1. Comparative adverse reactions and sow performance on each farm between EPL and other commercial vaccines. Different superscripts a, b) show statistically significant differences ($P < 0.05$).

Parameter	Farm 1		Farm 2	
	A	EPL	B	EPL
No. of sows	112	112	124	124
Local reaction (%)				
Redness at injection site	0.89	0	1.61	0
Swelling at injection site	2.68	0	0	0
Systemic reaction (%)				
Respiratory distress	1.78	0	5.65	0
Rash	14.3	2.68	8.87	0.81
Vomiting	2.68	0	1.61	0
Diarrhea	0	0	0	0
Sudden death	1.79	0	0	0
Average Feed intake at vaccination day (kg)	**5.14[a]**	**6.59[b]**	**5.26[a]**	**6.57[b]**
Adverse reactions (%)	18.8[a]	2.68[b]	8.87[a]	0.81[b]

Conclusions and Discussion

These results indicate that EPL is a safer vaccine for gilts and sows compared to vaccines A and B. Furthermore, the safety of these vaccines influences the average feed intake of the sow, which could cause a reduction in milk production and consequently, worse piglet performance at weaning, as seen in previous studies[3].

In future, not only reproductive vaccines should be evaluated by seroconversion of their respective antigens, but safety issues that can negatively affect piglet and sow performance should also be taken into consideration.

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Control of sow mortality by *Clostridium novyi* vaccination in Argentina

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Introduction

One of the most common problems faced in the field is the increase in sow mortality rates and therefore the economic impact that this represents. It is not unusual nowadays to find mortality rates that were considered unacceptable just a few years ago[1]. *Clostridium novyi* (*Cl.noyvi*) is an anaerobic, gram-positive and alpha toxin-producing microorganism that causes porcine infectious necrotic hepatitis[2]. Sudden death in sows is commonly seen during the peripartum period (near the end of gestation and during farrowing)[3]. The aim of this study was to evaluate the impact of *Cl.novyi* immunization by using a commercial vaccine as a tool for the prevention of sudden death.

Materials and methods

The present study was conducted on a farrow to finish farm of 1050 sows located in Entre Ríos, Argentina, after it had reported an increase in the incidence of sow mortality. The average annual mortality rate in 2016 reached 8.48%. *Cl.noyvi* was confirmed by liver samples sent to the DIAGNOS® Laboratory using FTA® ELUTE cards (Whatman Inc., Florham Park, NJ). A multiplex polymerase chain reaction test (PCR) was carried out and the presence of the genes encoding for *C.novyi* type B was detected. The entire farm was then vaccinated and revaccinated, following the manufacturer's instructions, with SUISENG®, an inactivated vaccine containing *E.coli* fimbrial adhesins, the LT toxin, *Clostridium perfringens* beta toxin and *Cl.novyi* alpha toxin. The sow mortality rate was compared between two different periods, namely Period A from January 2016 to April 2017, (non-vaccinated period) and Period B from May 2017 to December 2019 (SUISENG® vaccination). Finally, a Wilcoxon test was performed to compare the two periods.

Results

Based on the historical data, the average monthly mortality rate (Figure 1) was significantly decreased from 0.66% to 0.36% (Figure 2) (*p-value; 0.0021)* after the immunization of the herd. This represents a reduction of 45% in the mortality rate.

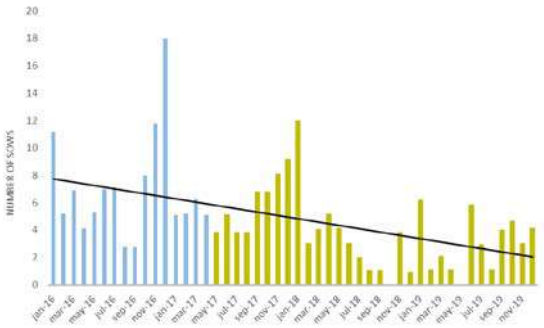

Figure 1. Progression of monthly mortality in Period A (blue) and Period B (green)

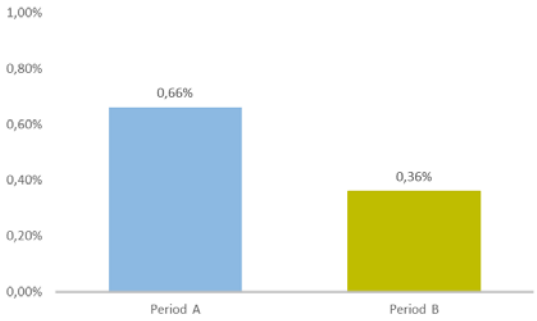

Figure.2: Comparison of the average monthly mortality rate between period A (blue) and period B (green).

Conclusions and Discussion

These results suggest that on this farm, *Clostridium novyi* was one of the main aetiological agents responsible for sow mortality. After the implementation of a vaccination programme against the alpha toxin, the mortality rate was significantly reduced[4]. It is noteworthy that good diagnostic tools to detect the presence of *C.novyi* form a key part of this process. Further studies should be performed to evaluate the role of *Clostridium novyi* in problems of sudden death in sows.

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Efficacy of different *Mycoplasma hyopneumoniae* bacterins against experimental *Mycoplasma hyopneumoniae* infection

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Introduction

Mycoplasma hyopneumoniae is the primary pathogen causing enzootic pneumonia (EP) in pigs and one of the primary agents involved in the porcine respiratory disease complex[1]. To date, vaccination against this pathogen is carried out worldwide[2]. However, vaccination triggers only partial protection with varying results between pigs and herds. It is believed that the adjuvant, which enhances the immunogenicity of the vaccine, also plays a role in both the local and systemic immune responses. Therefore, the present study was conducted to assess the protective efficacy of two different, commercial bacterins and the effect of an adjuvant (and immunostimulant) without an antigen using an experimental infection model.

Materials and Methods

Upon arrival at the experimental facilities, fifty-three 4-week old piglets that were free of *M. hyopneumoniae* were randomly divided into 5 groups: V1 Hyogen® (n=12), V2 another commercial bacterin (n=12), V3 same as V1 but without the antigen (n=12), PC non-vaccinated challenged control group (positive control) (n=12), NC non-vaccinated non-challenged control group (negative control) (n=5). After an acclimation period of 9 days, pigs in groups V1, V2 and V3 were vaccinated intramuscularly on D0, while the animals in the PC and NC group received a physiological saline solution intramuscularly. On D21, all animals except those in the negative control group were endotracheally inoculated with two different *M. hyopneumoniae* strains (two times 7 ml 10^7 CCU/ml per pig). Clinical signs were assessed daily using a respiratory disease score (RDS) (score 0 - 6) and blood samples were taken on D0, D7, D21, D35 and D49 to determine serum antibody levels with a blocking ELISA (IDEIA™ *Mycoplasma hyopneumoniae*, Oxoid) and *M. hyopneumoniae*-specific IgG levels using an in-house indirect ELISA (expressed as OD values). Animals were euthanized on D49, 4 weeks after challenge, and the macroscopic lung lesions (MLL) were evaluated (score 0 - 35).

Results

The results of the RDS, MLL and serology are shown in Table 1.

Table 1. Differences between the groups for RDS, MLL, Oxoid ELISA D21 and IgG levels on D21 and D49. (Mean ± standard deviation). Groups that have no superscript in common are significantly different from each other (P≤ 0.05).

	V1	V2	V3	PC	p-value
RDS (0-6)	$0.24^{a} \pm 0.43$	$0.60^{a} \pm 0.55$	$0.46^{a} \pm 0.32$	$0.60^{a} \pm 0.35$	> 0.05
MLL (0-35)	$0.05^{a} \pm 0.11$	$3.52^{b} \pm 4.10$	$4.52^{b} \pm 5.24$	$7.60^{b} \pm 5.15$	< 0.05
Oxoid D21	$0.60^{a} \pm 0.20$	$0.73^{a} \pm 0.18$	$1.69^{b} \pm 0.22$	$1.97^{b} \pm 0.59$	< 0.05
IgG D21	$0.14^{a} \pm 0.10$	$0.08^{ab} \pm 0.07$	$0.09^{ab} \pm 0.05$	$0.06^{b} \pm 0.03$	< 0.05
IgG D49	$0.91^{a} \pm 0.49$	$1.04^{a} \pm 0.58$	$0.41^{b} \pm 0.19$	$0.33^{b} \pm 0.16$	< 0.05

Conclusions and discussion

There were no significant differences in RDS, although the score was lowest in V1. There were significantly less macroscopic lung lesions in V1 compared to the other groups. Although at three weeks after vaccination, a significant serological response was detected for both bacterin groups (V1 and V2) with the Oxoid ELIS, only in V1 IgG levels were significantly higher compared to the non-vaccinated PC group. In the V3 group (only adjuvant), RDS (0.46 vs. 0.60) and MLL (4.52 vs. 7.60) were lower than in the PC, but the differences were not statistically significant.

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Field trial comparing two PCV 2 vaccines efficacy on average daily weight gain

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Introduction

The negative impact of PCV2 in the swine industry is well documented, as are the benefits of vaccination against the infection (1). One of the most commonly encountered benefits of PCV2 vaccination is the reduction of weight loss and thus improved average daily weight gain (ADWG) (2). Over the last 15 years several commercial vaccines have become commercially available. The purpose of this study was to compare the efficacy, in terms of weight gain, of two different vaccines; one (Ingelvac CircoFLEX) administered by intramuscular injection and the other (Porcilis PCV ID) using an intradermal injection device (IDAL).

Materials and methods

The study was carried out in a Danish commercial herd experiencing uneven production results in finishers. PCR analysis of blood samples from randomly selected finishing pigs showed moderate levels of PCV2.

The study was performed as a blinded, side by side trial according to a study protocol approved by the Danish Medicines Agency. The trial included 751 pigs vaccinated 2 weeks after weaning with one of the two vaccines. Pigs were randomly allocated to experimental groups by weight and tagged with electronic ear tags of the same colour in both groups to assure complete blinding. Groups were co-mingled within pens. The pigs were vaccinated by the farmer to mimic the field situation. Before vaccination, the pigs were marked with non-permanent spray colour to allow the staff to distinguish between the vaccination groups. Mean weight and ADWG was compared using Mann-Whitney U-test, and discrete data were analysed using Chi-square test with p=0.05 as the level of significance.

Table 1. Inclusion of pigs for the study (nursery).

Vaccination group	# pigs	Average weight	Stdev of weight
Ingelvac CircoFLEX®	375	10.4 kg	4.1 kg
Porcilis® PCV ID	376	10.4 kg	4.2 kg

Results

At vaccination, the average pig weight was 10.4 kg in both vaccination groups (Table 1). In the nursery, the average daily weight gain was even in the 2 groups and therefore, the groups still had statistically similar average weights when placed in the finishing unit (Table 2).
In the finishing period, the group vaccinated with Ingelvac CircoFLEX® had an average ADWG that was 11 g higher than the group vaccinated with Porcilis® PCV ID (p=0.088). That difference resulted in a mean finishing weight 939 g in advantage of the group vaccinated with Ingelvac CircoFLEX® (p=0.082) . In the group vaccinated with Porcilis® PCV ID there was a significantly higher number of pigs with a finishing ADWG below 950 g/day (p=0.038). During the trial, PCR analysis of blood and oral fluid samples revealed relatively low levels of PCV2 among finishing pigs.

Table 2. Productivity in finishers.

Average values for	Porcilis® PCV ID	Ingelvac CircoFLEX®
Weight in finishing	31.2 kg	31.3 kg
Weight out finishing	105.6 kg	106.5 kg
Total gain vaccination- slaughter	95.2 kg	96.1 kg

Discussion and conclusion

The results of this study suggest that, even with a relatively low PCV2 field challenge, there is a difference in the efficacy of the two commercial vaccines when applied under field conditions. Significantly more finishing pigs vaccinated with the intradermally administered vaccine gained less than 950 g/day; this resulted in a numerical difference of approximately 1 kg at slaughter in favour of pigs vaccinated with Ingelvac CircoFLEX®.

With the current pork price levels, 1 kg corresponds to €1.8 per pig (3). The economic and management advantage from each protocol needs to be considered when deciding which product to use on the farm.

Due to the study design, it was not possible to determine if the differences in performance observed were due to vaccine and/or the method of administration, or other factors. Since the vaccination process itself was not monitored during the study, incorrect IDAL administration technique cannot be excluded as a factor.

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Performance of Pigs Vaccinated With Porcilis™ Ileitis and Experimentally Infected With *L. intracellularis*

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Introduction

Lawsonia intracellularis is an obligatory intracellular bacterium that infects enterocytes causing the disease known as Proliferative Enteritis (PE). PE gross lesions are characterized by thickening of the intestinal mucosa due to enterocytes hyperplasia (2). Pigs vaccinated with the live attenuated oral vaccine are generally protected against PE, however, antimicrobials need to be suspended for three days before and after vaccination. The new injectable vaccine with the inactivated agent (Porcilis™ Ileitis, MSD), used in this study, is an alternative for controlling PE with no need to withdraw any medication (1). Therefore, the objective of the present study was to evaluate the performance of pigs vaccinated with Porcilis™ Ileitis.

Materials and Methods

Seventy-two 3-week-old pigs from a herd with no history of PE were used. All animals were housed in a research facility at UFMG. Animals were weighed and randomly homogenized in 4 rooms, with free access to water and non-medicated feed since arrival. Animals of each treatment group were allocated in different rooms: Vaccinated/Challenged (VAC/CHAL), Vaccinated (VAC), Challenged (CHAL) and Control (CONT). All animals were weighed on days 0, 28, 49 post vaccination. Animals from VAC/CHAL and CHAL groups were inoculated intragastrically with intestinal homogenate of PE sick pigs on day 28 post-vaccination (10^9 organisms of *L. intracellularis*). Feed consumption (supplied minus wasted) was recorded during the experiment from days 0-28 and 28-49.

Results

Average daily gain (ADG) and feed consumption (FC) results are summarized in tables 1 and2, respectively. In the period from day 0 to day 28, there was no difference in ADG and FC among all the experimental groups. From day 28 to day 49, for ADG and FC, a significant reduction was observed among the CHAL and the other groups.

Conclusions and Discussion

The result of ADG and FC in the period from day 28 to 49 demonstrated that the CHAL group was significantly affected by the disease with an average gain of 253.035 grams per day and the lowest feed consumption when compared to other groups. Vaccinated animals challenged with *L. intracellularis* performed as well as control animals.

Table 1. Average daily gain from groups Control (CONT), Vaccinated (VAC), Challenged (CHAL) and Vaccinated/Challenged (VAC/CHAL).

GROUP	ADG 0-28	ADG 28-49
CONT	177,57±55,77 a	430,54±126,52 a
VAC	156,25±63,20 a	387,14±214,03 a
CHAL	167,83±84,53 a	253,035±235,52 b
VAC/CHAL	175,46±65,30 a	442,35±159,64 a

Table 2. Feed consumption from groups Control (CONT), Vaccinated (VAC), Challenged (CHAL) and Vaccinated/Challenged (VAC/CHAL)

GROUP	FC 0-28	FC 28-49
CONT	2108,20 a	3802,28 a
VAC	2441,51 a	4017,20 a
CHAL	2323,39 a	2882,97 b
VAC/CHAL	2177,56 a	3907,32 a

Based on the ADG and FC, Porcilis™ Ileitis vaccined pigs demonstrated significantly improved results when compared to CHAL animals.

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Humoral and cell-mediate Immune Response Evaluation in Pigs Vaccinated With Porcilis™ Ileitis and Experimentally Infected with *L. intracellularis*

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Introduction

Lawsonia intracellularis is an obligatory intracellular bacterium that infects enterocytes causing the disease known as Proliferative Enteritis (PE). PE gross lesions are characterized by thickening of the intestinal mucosa (5). Protective immune response against *L. intracellularis* in pigs are commonly associated with the production of IgG and IgA antibodies in the serum and intestinal mucosa, respectively. In addition, there is an increase in the lymphocyte $CD8^+$ population and Interferon-γ secretion (1, 3, 4, 6). A new injectable vaccine with the inactivated agent was used to evaluate the development of humoral and cell-mediated immune response against *L. intracellularis*.

Materials and Methods

Seventy-two 3-week-old pigs from a herd with no history of PE were used. All animals were housed in a research facility at UFMG. Animals were weighed and randomly homogenized in 4 rooms, with free access to water and non-medicated feed since arrival. Each room received an experimental group: Vaccinated/Challenged (VAC/CHAL), Vaccinated (VAC), Challenged (CHAL) and Control (CONT). Serum samples were collected from all animals and tested by IPMA to detect specific serum IgG and ileal wash IgA specific against *L. intracellularis (2)*. Whole blood samples were collected from animals in the 4 groups and were tested by ELISPOT technique to detect IFN-γ producing cells that respond specifically to *L. intracellularis* antigen *(3).*

Results

Median of serum titers are shown in Table 1. Low (1:30) titers detected in a few animals on day 0 are likely maternal. There was an increase in active IgG production 14 days post-vaccination (dpv) in groups VAC and VAC/CHAL. The peak of serum anti-*L intracellularis* IgG detection occurred on day 28pv. On day 49pv, IgG specific to *L. intracellularis* was detected in all animals in the VAC and VAC/CHAL groups and in 4 animals in the CHAL group.

IgA anti-*L intracellularis* in ileal wash was detected in 8 of 16 animals in the VAC/CHAL group only on day 49pv. And the higher titer detected was 1:256.

Table1. Serum IgG anti-*L. intracellularis* titers detected on days 0, 14, 28 and 49 post-vaccination.

Group	0 dpv	14 dpv	28 dpv	49 dpv
CONT	0^a	0^a	0^a	0^a
VAC	0^a	$1:120^b$	$1:480^b$	$1:300^b$
CHAL	0^a	0^a	0^a	0^a
VAC/CHAL	0^a	$1:30^b$	$1:480^b$	$1:1480^b$

On day 49pv, VAC/CHAL animals had superior cell-mediated immune response when compared to CONT group (Table 2). The CHAL and VAC groups developed specific *L. intracellularis* cellular immune response on day 49, but they did not differ from the CONT or the VAC/CHAL groups.

Table 2. Mean of IFN-γ producing cells by the ELISPOT technique on days 0, 28 and 49pv.

Group	0 dpv	28 dpv	49 dpv
CONT	0^a	$0,35^a$	0^a
VAC	8^a	29^a	$47,9^{ab}$
CHAL	$0,5^a$	$10,5^a$	$16,33^{ab}$
VAC/CHAL	$2,2^a$	$39,8^a$	$82,6^b$

Conclusions and Discussion

Based on IPMA and ELISPOT results, the Porcilis™ Ileitis vaccine proved to be efficient in inducing specific humoral and cellular responses, detecting systemic IgG, mucosal IgA and systemic lymphocytes secretors of IFN-γ against *L. intracellularis* antigens.

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High quality Foot-and-Mouth Disease vaccines: its importance to ensure success in vaccination programs

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Introduction

Foot-and-mouth disease (FMD) is the most economically important disease of livestock because of its impact on international trade in animals and animal products. One key factor in the success of vaccination programs is the availability of high-quality vaccines. This study presents data regarding vaccine quality attributes and additional evidences of cross immunity against a wide spectrum of FMD virus (FMDV) (1).

Materials and Methods

The vaccines were produced by Biogénesis Bagó (Argentina) in accordance with good manufacturing practice as single water-in-oil emulsion (PD50>6) using purified antigen (O1 Campos/ A24 Cruzeiro/ A2001 Argentina). Final product testing is carried out on every batch before release for sterility, purity, safety and potency following the OIE Manual guidelines (2) and established national legislation. In addition, antigen content in every batch was monitored using HPLC as previously described (3). To assess duration of immunity, immune response after vaccination and revaccination was followed in pigs. Heterologous immunity was studied by OIE FMD Reference laboratory against a wide range of FMDV isolates belonging to topotypes/lineages currently circulating in Middle East and Asia.

Results

The assessment of antigen content in every batch by modern HPLC is a relevant indicator of consistency of production and vaccine potency. None of the batches of vaccines produced systemic or local reactions at the site of inoculation in the vaccinated pigs. Regarding potency in pigs, antibody titres above protection levels after vaccination revealed compliance with potency requirements of every batch. Long-term duration of immunity was shown in pigs by a single vaccination (6 months). Revaccination induced higher levels of antibodies which are required to ensure greater herd immunity. Regarding heterologous protection satisfactory vaccine matching results were achieved against relevant circulating strains belonging to the following topotypes/ lineages: O/CATHAY, O/SEA/Mya-98, O/ME-SA/PanAsia-2, O/ME-SA/PanAsia, O/ME-SA/Ind2001, O/EA-3 (Table 1), A/ASIA/Sea 97, A/ASIA/GVII, (r1 values >0.3) and high levels of neutralizing antibody titres. FMD vaccine should meet all quality standards during its shelf life (stability studies). The Biogénesis Bagó FMD vaccine showed antigenic stability for at least 24 months from its manufacturing date.

Table 1. r1 value of vaccine containing O1 Campos against field viruses (post vaccination pool serum)

Topotype/ lineage	Isolate	r1-value*
O/CATHAY	O/HKN/1/2013	1
O/SEA/Mya 98	O/SKR/84/YDM/2015	0,68
	O/TAI/1/2017	0,41
O/ME-SA/PanAsia	O/VIT/1/2018	0,68
	O/TAI/4/2018	0,56
O/ME-SA/PanAsia-2	O/VIT/1/2013	0,47
	O/IRN/12/2018	0,50
O/ME-SA/Ind2001	O/SKR/4/2019	0,44
O/EA-3	O/ALG/1/2018	0,37

*≥0.3 indicate that the field isolate is sufficiently similar to the vaccine strain so that use of a vaccine based on this strain is likely to confer protection against challenge with the field isolate

Conclusions and Discussion

High potency vaccines conferred heterologous immunity against a wide spectrum of circulating viruses. The value of assessing the capacity of vaccine being applied in the field in protecting against the circulating strains is emphasized. The application of multivalent vaccines of high assured quality among other sanitary measures is a key factor for the success of a FMD control and eradication program.

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Vaccination at two weeks of age with Ingelvac PRRSFLEX® EU in the presence of maternally derived antibodies (MDA) protects piglets against a heterologous PRRSV1 challenge

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Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS) Virus is one of the major pathogens in pigs that have a significant economic impact on the swine industry worldwide. Modified life vaccine (MLV) against PRRSV has been demonstrated as an effective tool to control clinical signs related to infection. In literature, it is described that maternally derived antibodies (MDA) might interfere with MLV vaccination efficacy.

Materials and methods

On a farm all sows of the breeding herd were vaccinated with ReproCyc PRRS® EU in a mass vaccination scheme. A total of 59 piglets were randomized in two groups, 30 piglets were vaccinated at two weeks of age, and 29 piglets remained untreated. At weaning piglets were transferred to a controlled laboratory environment. At 10 weeks of age piglets were challenged with a heterologous PRRSV1 strain and necropsied 10 days post challenge.

Blood was collected at birth, and at 2, 4, 6, 9, 10 weeks of age as well as at necropsy for serum (ELISA) and viremia (qPCR, necropsy only).

Lung tissue was collected at necropsy to detect viremia (qPCR). Lungs were investigated for PRRSv specific lesions and mean lung lesion scores were calculated.

Peripheral Blood Mononuclear Cells (PBMC) were collected before challenge and at necropsy and stimulated for a PRRSv specific Interferon (IFN)-γ response.

Results

Piglets of both groups were tested positive for MDA after colostrum intake. Mean antibody titers were declining over time in the untreated group and turned below the cutoff level of the test at 9 weeks of age. In contrast, the vaccinated piglets remained sero-positive throughout the time of challenge (1). After challenge both groups showed rising antibody levels with significantly higher levels in the vaccinated piglets ($p<0.0001$).

Mean lung lesion scores were calculated at necropsy. The vaccinated piglets showed a significantly reduced score compared to the non-vaccinated group ($p=0.0125$).

Viremia was measured in the blood and in lung tissue at time of necropsy. All piglets of the non-vaccinated group were tested positive by qPCR while only 90% of the vaccinated animals were tested positive 10 days post challenge. Vaccinated piglets showed a significantly reduced viral load both in serum and in lung tissue compared to the non-vaccinated piglets ($p<0.0465$).

Stimulation of PBMC before challenge showed no response to PRRSv specific stimulation in the non-vaccinated group while the vaccinated group showed a robust primary response. After challenge the non-vaccinated showed an immune response comparable to pre-challenge levels of the vaccinated group, however, the vaccinated group showed a significant raise in the number of PRRSv specific responding cells ($p=0.0052$).

Discussion

The transfer of maternally derived antibodies from sow to piglet has been shown to interfere with the antibody response to PRRSv vaccination (2). This study provided data that vaccination with Ingelvac PRRSFLEX® EU at two weeks of age is capable to induce a cellular and humoral immune response in the presence of MDA that leads to clinical protection by reducing viral load in tissue and serum as well as a reduction of lung lesions after controlled artificially challenge with a PRRSV1 strain. These results confirm previous experiences gathered in field situations were vaccinated piglets showed a significantly reduced viremia in a side-by-side study (3).

Conclusion

Ingelvac PRRSFLEX® EU is able to overcome limitations of other PRRSV1 MLV vaccines by providing protection against heterologous challenge in the presence of MDA.

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Efficacy of an inactivated Seneca Valley virus vaccine in pregnant sows

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Introduction

Senecavirus A (SVA), commonly known as Seneca Valley virus (SVV), is a causative agent of vesicular disease in swine that is clinically indistinguishable from foot-and-mouth disease (FMD). Due to the severe consequences of FMD entering a FMD-free country, a foreign animal disease investigation (FADI) must be performed every time a vesicular lesion is observed. These investigations have economic ramifications especially for the state and federal government. A large portion of the investigations have been centered at sow slaughter facilities, with some facilities requiring investigations weekly. Having an effective SVV vaccine would be a valuable tool for the swine industry to improve swine health and reduce disruptions related to foreign animal disease investigations.

Materials and Methods

Part 1: Pregnant sows were randomly placed into 4 groups: Group 1 (vax + challenge, n=9), Group 2 (vax + sham, n=3), Group 3 (no vax + challenge, n=5) and Group 4 (no vax + sham, n=3). All animals were negative for SVV neutralizing antibodies at the start of the study. Around 70 and 90 days of gestation Groups 1 and 2 were vaccinated intramuscularly (IM) with 2 mL of whole-virus inactivated SVV (2015 isolate) with an oil-in-water emulsion adjuvant. Sows were intranasally challenged around 104 days of gestation (0 dpc) with 5 mL of a heterologous 2018 SVV isolate (10^7 $TCID_{50}$/mL, challenge) or media (sham). Sows were rectal swabbed on 0, 1, 2, 3, 5, 7, 14, 21, and 28 days post challenge (dpc) and checked for vesicular lesions. Sows were bled on -35, -14, 0, 3, 7, 14, and 28 dpc.

Part 2: Piglets (3-6 days old) from Groups 2 and 4 were challenged orally with 2 mL of 2018 SVV (10^5 $TCID_{50}$/mL, challenge) to test lactogenic immunity. Piglets were bled and swabbed on 0, 5, 14, 24 dpc.

Swabs and serum were tested for SVV RNA by PCR and the antibody response was determined by virus neutralization (VN) assay.

Results

Part 1: In Group 1 (vax + challenge), all animals had VN titers between 1:64 and 1:1024 following 2 doses of vaccine. Following heterologous challenge, there were no clinical signs of vesicular disease. SVV RNA was not detected in serum or rectal swabs tested by PCR except for 1 rectal swab in 2 animals at 5 dpi. In addition, no anamnestic neutralizing antibody response was observed after challenge. In Group 2 (vax + sham), all animals developed a VN titer between 1:256 and 1:1024 following two doses of vaccine. After sham challenge, no animals had vesicular lesions and SVV RNA was never detected in serum or fecal samples. 2/5 Group 3 (no vax + challenge) animals did develop vesicular lesions after challenge and SVV RNA was detected in at least once serum or swab sampled tested. Four weeks after challenge, sows had VN titers that ranged from 1:1024 to 1:4096. Finally, Group 4 (no vax + sham) sows did not develop clinical signs, SVV RNA was not detected in samples tested and they did not develop neutralizing antibody titers.

Part 2: Piglets born to vaccinated sows (Group 2) were protected against SVV challenge. No SVV RNA was detected in tested serum and swab samples. In contrast, piglets born to non-vaccinated sows (Group 4) did develop viremia and many shed virus in feces detected by PCR after SVV challenge. Other than a mild transient diarrhea observed in a few piglets no other clinical signs were observed in challenged piglets.

Conclusions and Discussion

In these studies, a whole-virus inactivated SVV vaccine protected against clinical disease, prevented viremia, and reduced or eliminated virus shedding in feces in sows after heterologous challenge. Sows vaccinated during gestation produce lactogenic immunity that provided protection to piglets challenged during the first week of life. SVV is present in some slaughter plants and could be playing a role in the large number of FADIs occurring at sow slaughter plants. Unpublished work has also demonstrated efficacy of this vaccine in nursery-aged pigs. In addition, another group has shown protection from an inactivated SVV vaccine in finishing swine.[1] An effective SVV vaccine could have a positive impact on welfare in the swine industry and reduce the economic burden of foreign animal disease investigations especially at sow slaughter facilities.

Acknowledgments

Animal caretakers at NADC for assistance with animal husbandry and sampling. Deb Adolphson and Sarah Anderson for their technical expertise.

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Improvement of productive parameters after piglet PRRs vaccination

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Introduction

PRRS is an endemic swine disease causing significant productive and economic losses in pig farms[1]. Spain is one of the main global pig producers and, although sows' vaccination is consolidated, piglet's vaccination remains doubtful. However, it has been demonstrated that to vaccine piglets against PRRS brings some important benefits, like an improvement of productive parameters and a reduction of mortality[2]. The objective of this trial was to analyse how a PRRS positive farm improves its parameters after vaccination of piglets against PRRS.

Materials and methods

During December 2018-March 2019, a Site1+Site2 farm of 1.600 sows, located in Spain, experimented a huge mortality until the end of nursery (from 10 to 22%). PRRS positivity was confirmed on March 2019 through PCR on blood (2 pools/age, 5 animals/pool) at 4, 6, and 9 weeks of age (DIAGNOS, HIPRA, Spain). On April 2019, it was decided to start the piglet vaccination with UNISTRAIN® PRRS (HIPRA) with one intradermal shot at 2 weeks of age.

Animals were tested 8, 16 and 24 weeks after vaccination. 3 pools (5 animals/pool) at weaning and at the end of nursery were analysed by PCR on DIAGNOS (HIPRA, Spain). Additionally, at the end of nursery, the PRRS strain was also sequenced.

Results and discussion

On the one hand, after starting vaccination, all PCRs (at 8, 16 and 24 weeks) were negative at weaning, but positive at the end of the nursery. The sequencing confirmed that the strain detected after nursery was that corresponding to UNISTRAIN® PRRS. These results confirm that vaccination succeeded in reducing the virus circulation at weaning and also succeeded in replacing PRRS field strains.

On the other hand, mortality was statistically significant reduced from 16% to 4% (figure 1) and treatments were also reduced after UNISTRAIN® PRRS vaccination (figure 2).

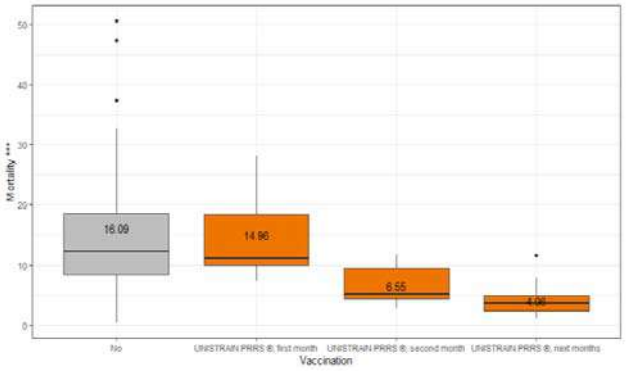

Figure 1. Mortality reduction after vaccination with UNISTRAIN® PRRS. ***Significant differences

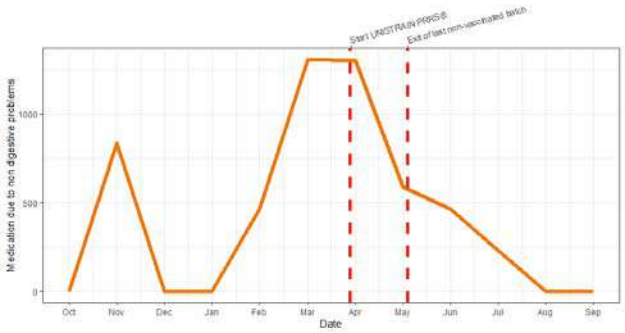

Figure 2. Antibiotic+anti-inflmamatories reduction after vaccination with UNISTRAIN® PRRS.

Conclusion

Piglet's vaccination helps to reduce PRRSV circulation in the farm, therefore improving performance and reducing economic losses during nursery.

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Evaluation of the field safety and efficacy of the combination of UNISTRAIN® PRRS and AUSKIPRA® GN in sows

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Introduction

Breeding sows are repeatedly vaccinated against several agents. To simplify complex immunization schedules, combined administration of vaccines is used. Advantages of combined vaccines are fewer injections, improved animal welfare and less stress to the animals, greater convenience, less work and a reduction in administration costs (1). Recently, the combination of UNISTRAIN® PRRS (MLV PRRSV1 vaccine, HIPRA) and AUSKIPRA® GN (pseudorabies live vaccine, HIPRA) has been licensed for intramuscular and intradermal administration (2 ml and 0.2 ml respectively). A previous study evaluating the field safety and efficacy of the combination has been published (2). However, safety of the vaccine combinations is still a big concern amongst Thai swine farmers. Hence, the aim of this study was to evaluate the safety and efficacy of this combination on commercial swine farms in Thailand.

Materials and Methods

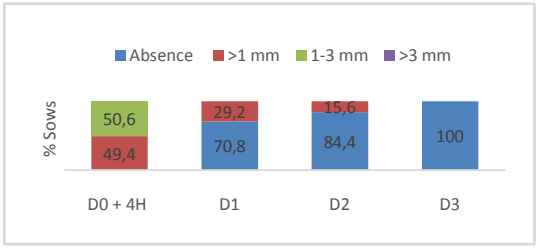
This study was carried out in 2018 on a 1,170-sow farrow to finish farm located in western Thailand. A PRRSV2 outbreak was confirmed by RT-PCR (Kasetsart University) and real time PCR (Diagnos HIPRA) positive results in serum. Recent PRRSV circulation in the grower-finisher pigs was also detected by paired serum tests (ELISA kit). The pre-weaning mortality rate in the first weeks of the outbreak was up to 25%. Sows were vaccinated intradermally with the combination (0.2 ml) applied with Hipradermic® following the vaccination schedule of the farm. The safety of the combination was based on the evidence of local and systemic adverse reactions after vaccination, monitoring it daily from day 0 until 14 days post vaccination (pv).The efficacy of the combination in controlling PRRS problems was determined by a 6-month period comparison of the reproductive and productive parameters before and after starting the use of the vaccine combination, (IBM SPSS statistics 22 program).

Results

Regarding the safety, no severe adverse reactions were observed in the vaccinated sows apart from the normal papule observed after the vaccination. Those sows with skin redness returned to normality one or two days pv without medical treatment (Fig.1 and 2). Significant differences were found in all the comparative parameters. (Table 1).

Discussion and conclusion

The combined administration of UNISTRAIN® PRRS and AUSKIPRA® GN was shown to be a useful tool for the control of PRRSV2 field infections, significantly increasing reproductive and productive parameters.



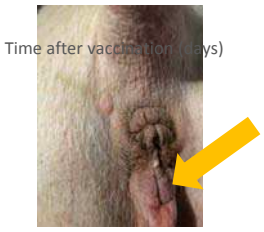


Figure 1 and Figure 2. Local reactions and an observed papule (orange arrow) in the sows after vaccination.

Table 1. Comparison of a 6 month period before and after using the vaccine combination UNISTRAIN® PRRS and AUSKIPRA® GN.

Parameter	Before	After	Diff
Farrowing rate (%)	72.8	91.4	+18.6
Abortion rate (%)	7.2	1.4	-5.8
Still births	2.1	1.1	-1
Mummification	1.9	0.4	-1.5
Live piglets	10.8	13.5	+2.7
ADWLG[1] (g/d)	1,687.5	3,026.3	+1,338.8
Pre-weaning mortality (%)	15.6	6.9	-8.7

All the parameters showed significant difference between groups ($p < 0.05$). [1]ADWLG: litter average daily weight gain in the lactation period.

Additionally, the combination administered intradermally with Hipradermic® is safe, increasing animal welfare and reducing the farmers' workload.

Acknowledgements

The authors would like to thank to pig farming cooperatives for providing the retrospective data and for their encouragement.

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Heterologous protection of a commercial Porcine Reproductive and Respiratory Syndrome Virus (PRRSV1) vaccine against PRRSV2 under field conditions in Taiwan

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Introduction Porcine Reproductive and Respiratory Syndrome virus (PRRSV) is ubiquitous in most swine producing countries around the world. Therefore, breeding herd stability, defined as the absence of vertical transmission of virus thereby producing PCR PRRSV-negative weaned piglets is the initial goal of a control program (1). Various Modified Live Virus (MLV) vaccines are commercially available, and several studies have shown not only good efficacy of MLV vaccines against challenge with homologous strains, but also partial protection against challenge with heterologous strains, whereas others have found poor cross-protection (2).
The purpose of this study was to evaluate the cross-protection efficacy of a commercial PRRS MLV vaccine containing PRRSV1 strain on a Taiwanese swine farm endemically infected with PRRSV2.

Materials and Methods A 700-sow commercial farrow-to-finish farm in the south of Taiwan was unstably PRRSV2-infected. After whole herd vaccination (Table 1) with PRRSV2 vaccine for 6 months, the sow herd was still unstable, as detected by quantitative PCR ($10^{4.58}$ copies/µl) in weaned piglets. Meanwhile, nursery pigs showed poor growth performance with a 66.5% survival rate. In August 2018, the PRRS vaccine was changed to a PRRSV1 vaccine (UNISTRAIN® PRRS, HIPRA).

Table 1. Whole herd vaccination program for PRRS

	Period	Breeding herd	Piglet
PRRSV2 vaccine	Feb-Aug 2018		
UNISTRAIN® PRRS	Aug 2018-Aug 2019	Mass vaccination 4times/year	7-10 days old

Based on the ORF5 sequencing, the wild virus circulating on this farm was 16% and 49.4% different respectively between the PRRSV2 and PRRSV1 vaccine. Phylogenetic tree showed the difference between the wild strain and the vaccine strains (Fig. 1). Vaccine efficacy was evaluated based on the stability of the sow herd, the growth performance and the viremia status during the nursery period. For this reason, the average survival rate in the nursery period was recorded and blood samples were taken from weaned piglets (3-4 weeks of age, WOA) and nursery pigs (6-7 WOA), quarterly in each case.

Results Sow herd PRRS stability was achieved after 3 months of PRRSV1 mass vaccination, maintaining a PCR-negative weaned piglet flow until the end of the study. In the nursery period, the rate of viraemic piglets decreased from 100% to 66%, with a decrease in the viral load from $10^{4.31}$ to $10^{2.16}$ from Aug. 2018 to Aug. 2019. Growth performance also improved during this phase, showing a decrease in the mortality rate from 34% to 5% (Fig. 2).

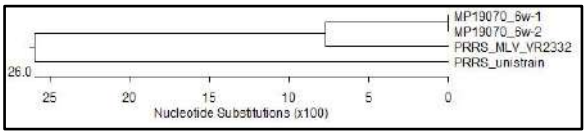

Figure 1. Phylogenetic tree for wild virus isolates and the 2 commercial PRRSV vaccines used in the study.

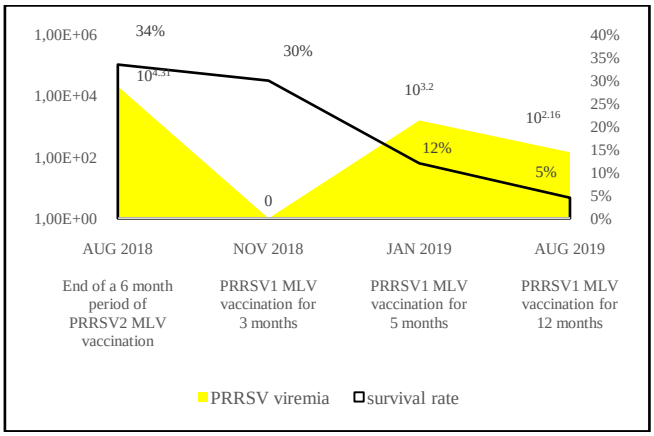

Figure 2. Yellow area: PRRSV viremia in nursery pigs (6-7 WOA). Black line: mortality rate during the nursery period (4-12 WOA).

Conclusions and Discussion
Heterologous protection conferred by UNISTRAIN® PRRS (PRRSV1 vaccine) against a field PRRSV2 infection was effective based on the outcome achieved, showing an improvement in productivity as a result of a reduction of the mortality rate during the nursery period, as well as keeping the sow herd PRRS-stable (PCR-negative weaned piglet flow until the end of the study).

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Emergency vaccination protocol with UNISTRAIN® PRRS after an outbreak of Porcine reproductive and respiratory syndrome on a negative swine farm in South Korea

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSv) is one of the diseases which causes the greatest economic losses to the swine industry. When PRRSv enters a negative farm cause a serious outbreak : increase of abortions over a period of months, weakborn piglets, an increase in pre-weaning mortality, etc. are often seen. Under these circumstances, minimisation of the impact of the disease is a primary objective. This can be achieved by emergency vaccination of sows with PRRS MLV vaccines. Vaccination with MLV vaccines has been reported to achieve farm stabilisation more quickly than other methods (1). This is a case report of a clinical outbreak on a negative farm in Korea.

Materials and Methods

A 450-sow breeding centre that was previously PRRS-negative was infected and this was confirmed by the laboratory by regular serum profile monitoring to detect PRRS antibodies (ELISA) and PRRSv (RT-PCR). As a measure to reduce the impact of the PRRSv outbreak, the farm decided to carry out emergency vaccination with UNISTRAIN® PRRS (0.2 ml), applied intradermally, using a Hipradermic® needle-free device. Mass vaccination of the sows was performed once per month, starting just after PRRSv confirmation and for 4 months after the outbreak. Herd closure, infected nursery depopulation and an exhaustive monitoring programme were other measures that were taken. The monitoring programme consisted of a serum profile for antibody detection (ELISA) once a month and oral fluid collection for PRRSv detection (RT-PCR) after the 4th vaccination in sows, suckling piglets, weaned piglets and fattening pigs (Figure 1). Moreover, all the housing materials were dismantled, cleaned and disinfected (Figure 2). High internal and external biosecurity measures were implemented. Feeding immune boosters (PMCplus®) after the 4th vaccination and antibiotic treatments (tylvalosin, bacitracin) were used to minimise co-infections. Sentinel pigs that were PRRSv ELISA and PCR-negative were introduced into the nursery units 7 months after the PRRSv outbreak and they were sampled by ELISA and PCR 3 weeks after entering the nurseries.

Results

The ELISA and PCR results from the time of the outbreak onwards are shown in Table 1. All animal categories were positive for ELISA and PCR until 2 months after the outbreak. 7 months after the outbreak, the farm started to become stable with PCR-negative results in the sow herd and negative ELISA and PCR results in the suckling piglets. 3 weeks after entering the nurseries, sentinel pigs were also negative for ELISA and PCR.

Table 1. ELISA and RT-PCR results for the detection of PRRSv antibodies (Ab) and PRRSv antigen (Ag).

	PRRS Outbreak		2 months after outbreak*		7 months after outbreak**	
	Ag	**Ab**	**Ag**	**Ab**	**Ag**	**Ab**
Sow	+ PRRSv1	+	+ PRRSv1	+	-	+
Suckling piglets	+ PRRSv1	+	+ PRRSv1	+	-	-
Weaning piglets	+ PRRSv1	+	x	x	-	-
Growing pigs	+ PRRSv1	+	x	x	x	x

*Start of nursery depopulation, ** Introduction of sentinel pigs. Results in yellow boxes correspond to sentinel pig sampling 3 weeks after they entered the nurseries. + (positive), - (negative), x (no pig population exists).

Conclusion

Amongst other measures, emergency vaccination with UNISTRAIN® PRRS on a previously negative farm helped to reduce the disease impact and to stabilise the farm after the PRRSv outbreak.

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Improving piglet performance with a PRRSV1 vaccine under field conditions in the Philippines

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Introduction PRRS is a swine disease with a very important economic impact on the swine industry (1). The huge economic and productive losses due to its endemic distribution and the high levels of mortality caused by both types (PRRSV1 and PRRSV2) makes the immunization of pigs a necessity in order to minimize the impact on affected farms (2). Immunization by modified live vaccines (MLV) has proved to be effective in controlling PRRSv1 and PRRSv2 infection (3). The purpose of this study was to evaluate the field efficacy of UNISTRAIN® PRRS (MLV PRRSV1, HIPRA, Spain) against PRRSv2 field infection and to determine whether there were differences in terms of piglet performance between UNISTRAIN® PRRS and a commercial MLV PRRSv2 vaccine.

Materials and Methods
184 piglets from a 500-sow commercial farrow-to-finish farm in the south of the Philippines positive for PRRSv2, were randomly selected and divided into two groups within litters (92 piglets per group).
Group A was vaccinated with UNISTRAIN® PRRS (2 ml) and group B was vaccinated with a commercial MLV PRRSv2 (2ml). Both groups were vaccinated at 18 days of age (DOA). Blood samples were collected at 2,8,12 and 19 weeks of age (WOA) to perform ELISA and PCR tests. The efficacy of the vaccine was studied by the piglets' performance (weight at birth, at weaning, at 75 days of age and at slaughter) and the mortality rate during the nursery period. Moreover, the cost of antibiotic treatments was measured throughout the study duration as an extra measure.

Results No differences were found between groups in the ELISA titres. As regards the PCR results, whilst group A was already negative for PCR from 8 WOA onwards until the end of the study, group B was still PCR positive at 8 and at 12 WOA until 19 WOA when it became negative. These results show a longer shedding period after vaccination of the PRRSV2 vaccine compared with UNISTRAIN® PRRS (Table 1).

Table 1. PCR results from blood samples extracted at 2,8,12 and 19 WOA.

	PCR result			
	2 weeks	**8 weeks**	**12 weeks**	**19 weeks**
Group A	Negative	Negative	Negative	Negative
Group B	Negative	Positive	Positive	Negative

With regard to piglet performance, no differences were found between groups in terms of weight. As for the mortality rate during the nursery period, this was higher in group B (10.87%) compared to group A (3.26%) with a significant difference between the groups (7.61%). (Fig 2).

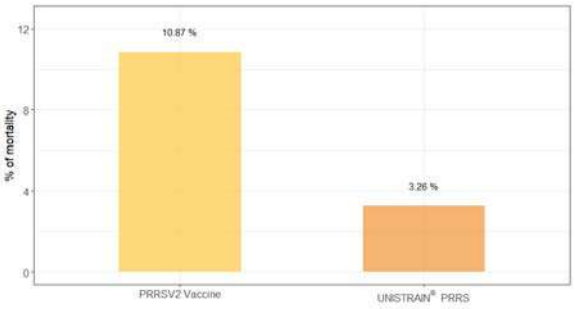

Fig 2. Mortality rate during the nursery period. p=0.08

Assuming an average loss of 3,500 Philippine pesos (PHP) per each dead animal during the nursery period, the decrease in mortality could result in a PHP 25,000 benefit during the period of the study.

The cost of the antibiotic treatments within the nursery unit during the study period was PHP 412.5 in group A whilst in group B it was PHP 519.6.

Conclusions and Discussion
Efficacy conferred by UNISTRAIN® PRRS (PRRSv1 vaccine) against a field PRRSv2 infection in piglets was effective based on the outcome achieved, showing an improvement in piglet performance as a result of a reduction in the mortality rate during the nursery period, as well as a shortening of the shedding period,

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Humoral immune response on different PRRS MLV vaccines in piglets

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Introduction

Porcine Reproductive and Respiratory Syndrome Virus is the causative agent of a disease that affects pigs worldwide and produces large economic losses[1]. Piglet immunization against PRRS with modified live virus (MLV) vaccines is being increasingly used in commercial farms. The objective was to describe the differences in humoral immune response after vaccination with different MLV vaccines.

Materials and Methods

Four farrow to finish farms with 300, 950, 1600 and 4000 sows respectively, in The Netherlands, switched piglet vaccination from either Porcilis® PRRS or Ingelvac PRRSFlex® EU at approximately 3 weeks of age to Suvaxyn® PRRS MLV around 2-3 days of age. In all 4 farms 2 consecutive batches were monitored, 1 batch vaccinated with either Porcilis PRRS or Ingelvac PRRSFlex and 1 batch vaccinated with Suvaxyn PRRS MLV. From each batch, 40 piglets were ear tagged and bled on day of vaccination (DV) and 6 weeks later. Antibodies were determined using IDEXX ELISA on all individual samples per batch (40 piglets) and IPMA (Gent University[2]) was performed in 40 samples per batch (20 piglets). Samples were pooled in 5 and investigated by PRRS PCR to determine the presence of virus on DV and 6 weeks later. If pools tested positive by PCR, it was tested by Bio-T kit ® PRRSV DIVA PCR for batches vaccinated with Suvaxyn PRRS MLV or ORF5-sequencing for batches vaccinated with Porcilis PRRS or Ingelvac PRRSFlex in order to differentiate field virus from vaccine virus.

Results

Field PRRSv was found in the 950-sow herd piglets in the Suvaxyn PRRS MLV group at DV and therefore this farm was excluded. Piglets vaccinated with Suvaxyn PRRS MLV in the other farms had higher maternally derived antibodies (MDA) at DV than Porcilis PRRS or Ingelvac PRRSFlex groups at DV measured by ELISA (Suvaxyn: mean=1.5, SD=0.6, Porcilis PRRS or PRRSFlex: mean=1.1, SD=0.5) and IPMA (Suvaxyn: mean=3.76, SD=1.41, Porcilis PRRS or PRRSFlex: mean=3.12, SD=1.83)(Fig.1 and 2). Humoral responses on vaccination are lower and show higher variation in the Porcilis PRRS or PRRSFlex groups than in the Suvaxyn PRRS MLV groups in ELISA (Suvaxyn:mean = 1.4, SD = 0.5, Porcilis PRRS or PRRSFlex:mean = 1.0, SD = 0.7) and IPMA (Suvaxyn:mean=5.91, SD=1.15, Porcilis PRRS or PRRSFlex:mean=3.55, SD=2.86).

In the Porcilis PRRS and PRRSFlex EU vaccinated batches a large number of individual animals showed no humoral response to vaccination at all in IPMA (42% (16/35) v.s. 25% (4/20) respectively) and ELISA (41% (32/78) v.s. 38% (15/40) respectively). Where in the Suvaxyn PRRS MLV vaccinated batches only a few individual animals showed no humoral response in IPMA (4% (2/51) and in ELISA (13% (14/109).

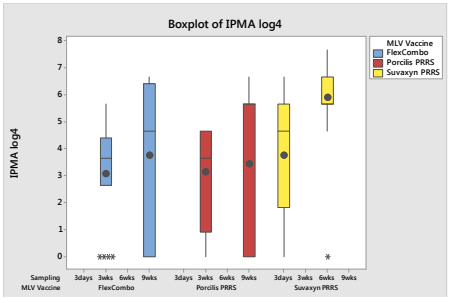

Figure 1. Boxplot of IPMA titers of vaccinated batches on DV and 6 weeks later.

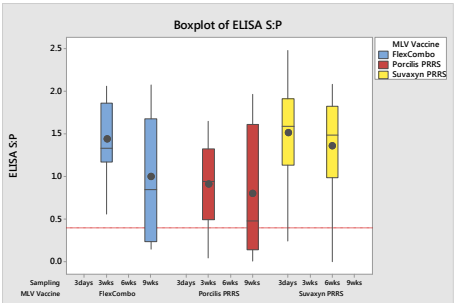

Figure 2. Boxplot of ELSIA titers of vaccinated batches on DV and 6 weeks later.

Conclusions and Discussion

After vaccination of piglets with Suvaxyn PRRS MLV around 2-3 days of age nearly all piglets showed a humoral immune response to vaccination, in contrast to vaccination of piglets with both Porcilis PRRS and Ingelvac PRRSFlex on 3 weeks of age where 1/4 to 1/2 of the animals doesn't show a humoral response to vaccination. Despite there is no evidence that no humoral response means no protection, further investigations should be performed to better understand these observations. Furthermore the results of this study showed that after vaccination of piglets with Suvaxyn PRRS MLV in presence of high levels of MDA, piglets show a serological response to vaccination.

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Effect of freshly mixed combination and ready-to-use PCV2 and *Mycoplasma hyopneumoniae* vaccines on productivity parameters in continuous pig flow in commercial pig farm

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Introduction

In recent years, vaccination against PCV2 and *Mycoplasma hyopneumoniae* (M.hyo) became routine measure in pig production worldwide. Besides traditional monovalent vaccines, ready-to-use (RTU) PCV2-M.hyo vaccines have appeared on the market (Best P., 2014). The aim of this study was the compare main production parameters in continuous pig flow of commercial pig farm after using freshly mixed monovalent vaccines and ready-to-use PCV2 and M.hyo vaccines.

Materials and Methods

The study was conducted in a one-site farrow-to-finish farm (1,700 sows). The herd was seropositive to PCV2, M.hyo, PRRSV-1 and APP. According to standard protocol, pigs were vaccinated at 21 days of age with a commercial PCV2, M.hyo and PRRS MLV vaccines. Pigs in group 1 (13,283 in 16 batches) were vaccinated once intramuscularly at 21 days of age with freshly mixed vaccines Ingelvac CircoFLEX® and Ingelvac MycoFLEX® (2 ml, FLEXcombo, Boehringer Ingelheim). Pigs in treatment group 2 (12,086 pigs in 14 batches) were vaccinated intramuscularly once at 21 days of age with a commercial ready-to-use vaccine containing inactivated PCV1-2 chimeric virus and M.hyo bacterin (2 ml, RTU). Pigs of one batch were assigned to the same treatment with alternate treatments between consecutive batches over a period of 7 month (December-July). Batches were kept in different rooms, but under the same management conditions in the same buildings. Lung check results performed according to PigMon and SPES. The Chi-square test was applied to analyse the results.

Results and Discussion

The trial results are presented in tables 1 and 2. Statistical significant difference could be demonstrated for mortality in nursery (3.30% vs. 5.15%, X2=53.96) and total losses for wean-to-finish (6.13% vs. 8.13%, X2=38.35) in favor of group 1 vaccinated with freshly mixed vaccines. FLEXcombo vaccinated pigs showed significant less M.hyo-like lung lesions and lower severity of pleurisy (APP Index) in SPES.

Table 1. Mean productivity parameters on nursery and finishing sites.

PARAMETERS	FLEXcombo	RTU
Nursery 31-89 days		
Batches, n	16	14
Pigs, n	13,283	12,086
Av. weight-in, kg	8.19±0.71	8.16±0.68
Av. age, days	31.1±1.1	31.5±1.2
Dead pigs, n (%)	439 (3.30%)[a]	623 (5.15%)[b]
Finishing 90-181 days		
Batches, n	16	14
Pigs, n	12,844	11,463
Av. weight-in, kg	37.00±2.47	36.26±4.44
Av. age, days	90.1±2.0	90.5±2.7
Dead pigs, n (%)	375 (2.92%)[a]	359 (3.13%)[a]
Total losses 30-181 days, n	814 (6.13%)[a]	982 (8.13%)[b]
Finish live weight	111.59±3.54	109.56±4.11
Finish age, days	181.1±1.5	181.3±2.1

(a, b) Superscripts indicate statistically significant differences (X2>3.84).

Table 2. Lung check results according to PigMon and SPES (pigs slaughtered in July).

PARAMETERS	FLEXcombo	RTU
Lungs, n	200	220
Average % of affected surface out of all lungs	2.10	4.41
Average % of affected surface out of lungs with active pneumonia	3.85	5.08
Lungs with Mhyo-like lesions ≥5% of surface out	28 (14%)[a]	68 (30.9%)[b]
Pleurisy 2-4 points in SPES, n (%)	42 (21%)[a]	73 (33.2%)[b]
APP Index	0.50	0.80

Conclusion

In this study the monovalent freshly mixed vaccines demonstrated some advantages over RTU. Vaccination with FLEXcombo resulted in additional two slaughtered pigs for every 100 vaccinated pigs compared to RTU.

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Characterization of the immune response to *porcine epidemic diarrhea virus* (S-INDEL European strain) in an experimental homologous challenge

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Introduction

Porcine epidemic diarrhea virus (PEDV) re-emerged in US in 2013 causing the death of millions of piglets (1). In Europe, less severe outbreaks have been described since 2014 (2). Depending on the presence of insertions and a deletion in the S gene, strains have been differentiated as S-INDEL (low virulence) or non-S INDEL (high virulence). Strains involved in European outbreaks correspond to the S-INDEL type, while both low and high virulence strains can be found in Asia and US. Regardless of virulence, PEDV may recur in a given farm after several months of clinical absence. The aim of the present study was to characterize the immune responses after an oral inoculation with a European S-INDEL strain and to evaluate the protection against a homologous challenge in a five-month lapse.

Materials and Methods

Ninety three-week old PEDV-negative piglets were divided in two groups: challenge (CH; n=75) and control (Co; n=14). One week later, piglets in CH were orally inoculated -0 post-infection (0 dpi)- with a PEDV S-INDEL European strain (2ml/animal; 10^4 $TCID_{50}$/ml). Five months later (154 dpi), both groups were inoculated as described above. Feces and sera samples were weekly collected after the first challenge, and immediately before the second challenge and at 157, 161, 164, and 168 dpi. Individual feces appearance was recorded. Samples were analyzed as follows: presence of PEDV using a commercial RT-qPCR (feces) and measure of antibodies (sera) against the spike glycoprotein using a commercial ELISA (IgG) and an in-house ELISA (IgA). Peripheral blood mononuclear cells (PBMC) and lymphocytes from mesenteric lymphnode (LML) were recovered immediately before and after the second challenge to measure systemic (PBMC) and local (LML) PEDV-specific IFN-g-secreting cells (SC) frequency by ELISPOT.

Results

Challenge at 0 dpi. During the first week, loose stools/diarrhea were detected in all animals from CH. In 9.5% of the animals, these findings were observed up to 21 dpi. PEDV was detected in feces in all challenged pigs (Table 1). Regarding antibodies, PEDV-specific IgG and IgA development had a similar pattern. Both IgG and IgA were constantly detected from 21 dpi in all animals and decline later until 154dpi (Figure 1). At that time, only 27% of pigs were still positive for IgG.

Table 1. Percentage of positive pigs and mean Ct for positive pigs by RT-qPCR (feces).

	7dpi	14dpi	21dpi	28dpi	35dpi
CH	100% 24.3	58.3% 32.6	28.8% 35.2	11.3% 33.4	7.4% 36.5
	154dpi	157dpi	161dpi	164dpi	168dpi
CH	0% -	84.7% 32.1	87.0% 27.3	37.0% 34.7	27.8% 34.9
Co	0% -	100% 27.8	100% 23.1	100% 31.0	50% 32.0

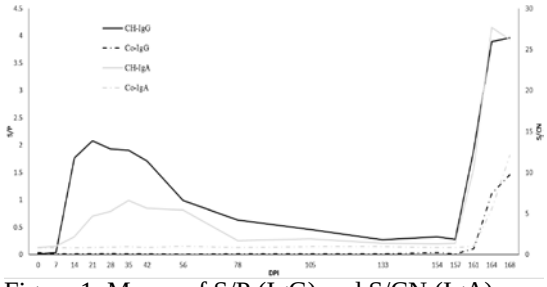

Figure 1. Means of S/P (IgG) and S/CN (IgA).

Homologous challenge at 154dpi. After the second challenge, PEDV was detected in all pigs from both groups, although not all the animals showed diarrhea (33.9% in CH and 80% in Co) and it did not extend beyond 3 days. Regarding humoral immunity, significant increases ($P<0.05$) were observed in CH for both IgG and IgA ELISA (Figure 1). For IFN-g ELISPOT, significant increases ($P<0.05$) were also observed in both PBMC and LML: from mean of 8.5 ± 3.2 specific-PEDV / IFN-g-SC million PBMC at 154 dpi to 18.0 ± 9.9 at 157 dpi; and from mean of 11.4 ± 6.6 specific-PEDV / IFN-g-SC million LML at 154 to 28.2 ± 13.9 at 157dpi. The boosters in terms of antibodies and cell-mediated immunity demonstrated that immunological memory was developed in group CH.

Conclusions

PEDV S-INDEL strain infection at four weeks of age induced immunological memory; however, the intensity of that immunity was not able to control a homologous infection, and the consequent excretion, in a five-month lapse. It seems that a homologous reinfection at older ages can occur even with no clear clinical signs. Further investigation concerning the role of these animals in the maintenance in the farm of the infection is needed.

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Efficacy of vaccination against early *Mycoplasma hyopneumoniae* infections

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Introduction

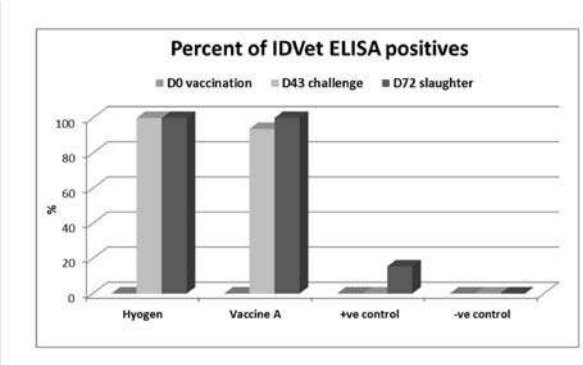
Mycoplasma hyopneumoniae (M.h.) is a primary pathogen of lungs and one of major agents responsible for Porcine Respiratory Disease Complex (PRDC), which causes substantial losses to the swine industry. Pigs of mid-finishing to slaughter age are mainly affected by PRDC. In some cases the circulation of the pathogen starts already in nursery. The aim of this study was to compare Hyogen® with another vaccine in the control of such early M.hyo infection.

Materials and methods

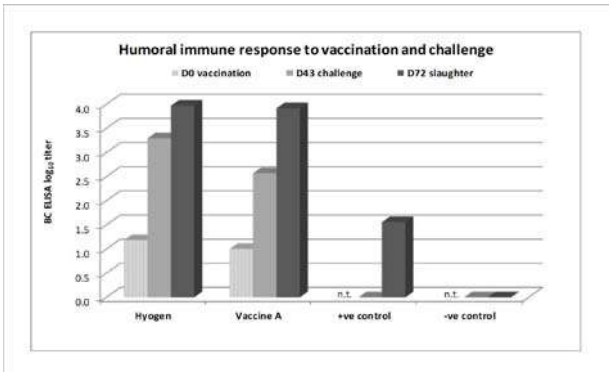
1 week old piglets were vaccinated either with Hyogen® (inactivated *M.hyo* and Imuvant™), Ceva or with Vaccine A (inactivated *M.hyo* and Amphigen®), or not vaccinated. At 7 woa challenge groups were inoculated intratracheally with different *M.hyo* strains on three consecutive days. Four weeks later the pigs were slaughtered and the lung lesions scored according to the European Pharmacopoeia 9.0. In questionable cases lung samples were collected for PCR and histopathology to confirm the specificity of the lesion. Blood samples were collected for M. hyo serology (IDVet ELISA) before vaccination, before challenge and at slaughter

Results

Hyogen vaccination induced stronger humoral immune response than vaccine A before the challenge (100% vs 95% positives and 3,3 vs 2,6 log BC titers ($p<0,05$).

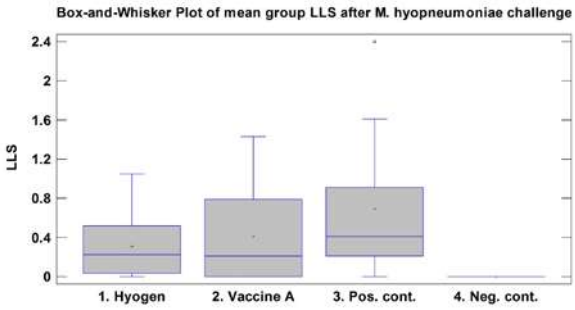


Graph 1. Percent of seropositive pigs per group.



Graph 2. Mean titers of M.hyo antibodies per group.

Further, Hyogen® vaccination lead to numerically lower mean LLS (0.3) compared to Vaccine A (0.4) and significantly lower than the positive (non-vaccinated challenged) control (0,7; $p<0,05$). Vaccine A lead to numerically yet not significantly lower LLS compared to the positive control.



Graph 3.

Conclusion

Hyogen® vaccination, contrary to Vaccine A provided significant protection against the development of lung lesions compared to the non-vaccinated challenged group in the early and massive *M.hyo* infection model. Its use can substantially aid the control of even early M.hyo infections in swine farms.

Efficacy of different combined or simultaneously administered vaccines against *Mycoplasma hyopneumoniae* infection

I.Kiss[1], Edit Kovács, Balázs Felföldi, Mihály Albert[2], Han Smits[1], Roman Krejci[3]

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Introduction

Enzootic pneumonia due to primarily *Mycoplasma hyopneumoniae* (M.hyo) and PCVD due to PCV2 virus remain a severe health and economic problem in pig farms. Vaccination against those two pathogens helps to reduce their clinical manifestation and corresponding losses. Several commercial mono- or bi-valent vaccines are available. The aim of this study was to evaluate the efficacy of different PCV2 and M.hyo vaccines in the experimental M.hyo challenge models.

Material and Methods

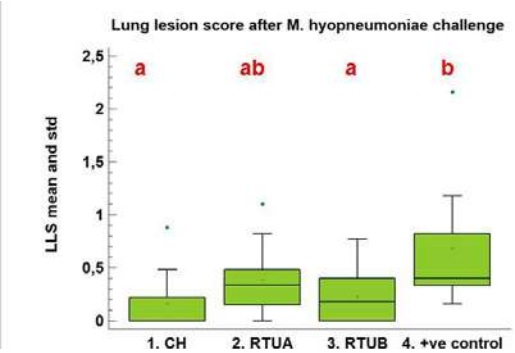
In two different experiments three-weeks old piglets were vaccinated either with Circovac® plus Hyogen®(CH) - both Ceva simultaneously or various PCV2+M.hyo RTU or RTM vaccines. In the trial 1) vaccines RTUA and RTUB were used and pigs were challenged at 7 weeks of age(WOA). In the trial2) vaccines RTUA and RTMC were used and the challenge was performed at 12WOA. Serum samples were collected prior to vaccination, challenge, and slaughter, and measured by BioChek and IDVet Mycoplasma hyopneumoniae ELISA kits. Pigs were always euthanized 4weeks later and lung lesions scored according to the European Pharmacopoeia 9.0. Doubtful macroscopic lesions were further investigated by histopathology.

Results

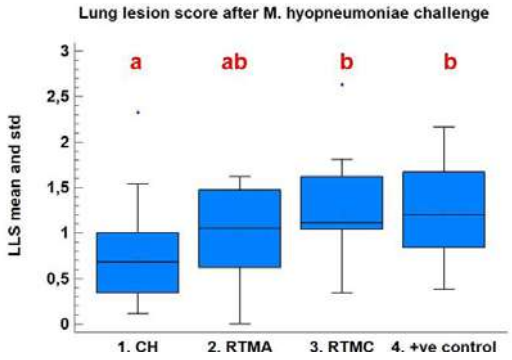
In both trials CH induced always higher M.hyo seroconversion prior to challenge than any other vaccines. Group mean lung lesion scores (LLS) in groups CH, RTU A, RTU B and positive control in trial 1)were as follows: 0.16; 0.38; 0.23; and 0.68. Only CH and RTUB differed significantly from the control.

Histopathology confirmed the macroscopic scores.

In the trial 2) the results for CH, RTUA and RTM and positive control were as follows 0.72; 0.98; 1,2; and 1.22, respectively.



Graph 1: Lung lesion scores in trial 1)



Graph 1: Lung lesion scores in trial 2.

Conclusion

This study demonstrated that Hyogen® administered simultaneously with Circovac® outperformed the already combined PCV2+M.hyo RTU and also the RTM vaccines concerning the protection against the development of lung lesions due to M.hyo. Some of the combined PCV2 and M.hyo vaccine may provide sub-optimal protection against M.hyo infection and thus the convenience of such use doesn't correspond with the expected efficacy.

Comparative potency of Circovac to induce seroconversion in gilts

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Introduction

Porcine circovirus 2 (PCV2) remains circulating in many sow herds, which represents the risk for clinical or subclinical PCV2-reproductive disease and/or early infections of newborn and suckling piglets. Circovac®, Ceva is the licensed PCV2 vaccine indicated not only for piglets but also for breeding female pigs. The aim of the trial was to compare its potency in comparison with another vaccine allowed to use in gilts or sows.

Materials and methods

The potency to induce the humoral immunological response was assessed by measuring the seroconversion in naïve gilts. In total 21 gilts were divided into three groups by 7 individuals. They were vaccinated 5 and 2 weeks before expected farrowing with either 2ml of Circovac, or 1ml of Vaccine A or not vaccinated as controls. The serum samples were individually collected before each vaccination and 2 weeks after booster vaccination. All samples were analyzed by quantitative Serelisa®PCV2 Ab Mono Blocking commercial ELISA. Each sample was tested in triple dilution quantitative analysis where the final result titer had to be calculated.

For the statistical evaluation the titers >15000 were considered as 15000. Wilcoxon's test was used to compare the set of data.

Results

All serum samples were negative before vaccination. The control group gilts stayed negative along all the observation period. All vaccinated animals reacted by the production of PCV2 specific antibodies already after primo-vaccination. In the Circovac® group 71% of animals after first vaccination and 86% after booster reached the titers >15000, while in Vaccine A group the maximal values after priming was 6467 and after booster 7386. The median titers in Circovac® vaccinated gilts were significantly higher than in Vaccine A group ($p<0,001$).

Tab 1.Median values of PCV2 antibody titers.

	Circovac	Vaccine A
After first vaccination	15000	3594
After second vaccination	15000	5028

Conclusions

The results of this confirmed a potency of Circovac® in inducing strong immune response shortly after the vaccination. Homogenous and high titers of PCV2 specific antibodies exceeded largely the responses after priming and booster induced by the Vaccine A. High levels of PCV2 antibodies are necessary to transmit the passive immunity to offsprings of vaccinated mothers in order to protect them against the field infections early in life. As demonstrated also previously Circovac® 2ml vaccination of sows provided stronger protection to piglets via passive immunity than another PCV2 vaccine administered to sows using its dose indicated normally for piglets[1].

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In-field evaluation of the efficacy of M.hyo and PCV2 vaccines in comparison with a vaccine combining both valences

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Ceva, France.

Introduction

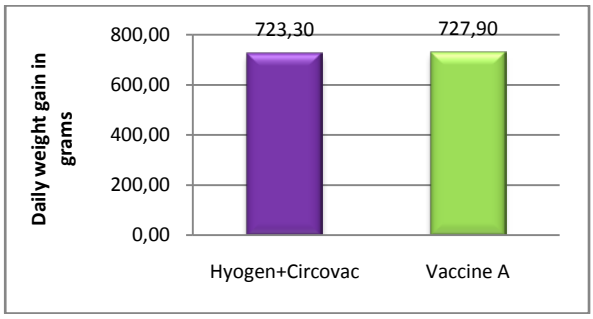
Hyogen® and Circovac® are inactivated vaccines against M.hyo and PCV2 and their efficacy was described in multiple studies. The aim of this trial was to compare their efficacy when injected simultaneously with a ready-to-use (RTU) PCV2+M.hyo vaccine.

Materials and methods

A commercial 2 sites swine farm with the history of EP and PCVD in Brittany, France, was selected for the trial. In total, from 3 batches, 418 piglets (G1) were vaccinated at 21 days of age (DOA) simultaneously with Circovac® and Hyogen®, 423 piglets (G2) with Vaccine A (PCV2+M.hyo RTU) and 30 piglets (G3) were not vaccinated. Mortality, weight gain, EP-like lung lesions using Ceva Lung Program method and PCV2 with M.hyo infection dynamics (using qPCR on tracheobronchial mucus collection) were examined and compared.

Results

Considering the pigs from groups 1 and 2: the weaning-slaughter mortality didn't differ statistically between G1 and G2 (4.31% vs 4.9%). No dead animal expressed PCVD specific lesions during necropsy. The weight gain was almost equal in G1 and G2 (723.3g vs 727.9g), p>0.05.



Graph 1. Average daily weight gain (g).

The average lung lesions score was 3.2/24 in G1; 4,2/24 in G2. The percentage of lungs with the scores >8 was lower in G1(16%) vs G2(27%), p<0.05. M.hyo loads in lungs was lower in vaccinated pigs compared to control especially at 21 WOA (3.73 in G1; 4.00 in G2 and 5.60 in G3–all as Log10).

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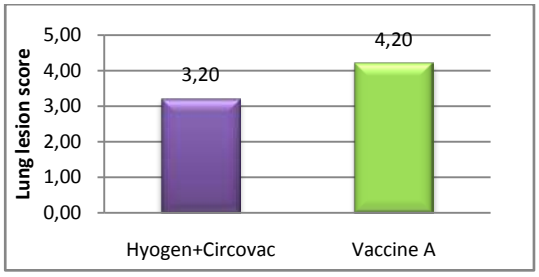
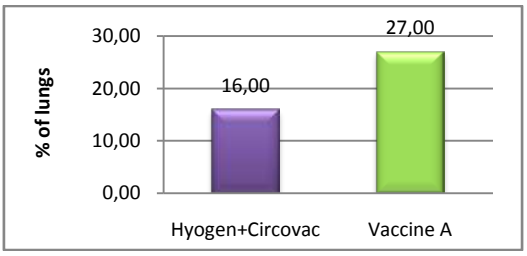
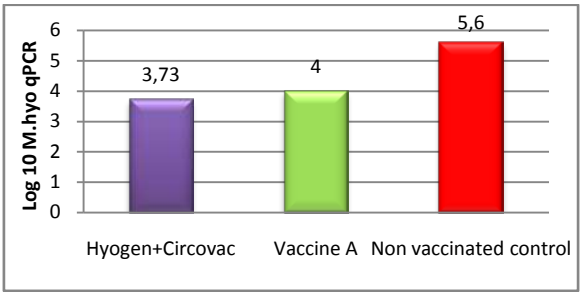


Graph2. EP-like lesion scores.



Graph 3. Percent of lungs with score >8.



Graph 4. M.hyo loads in lungs at 21 WOA.

Conclusion

In the described study, Circovac® and Hyogen® provided efficient protection against PCV2 and M.hyo infections, which was similar to PCV2+Mhyo combined vaccine. Circulation of both PCV2 and M.hyo was confirmed on the tested farm and was reduced in both vaccinated groups. Reduction of EP-like lung lesions was stronger in pigs vaccinated simultaneously with Circovac® and Hyogen®.

Field efficacy study of two different vaccination protocols against PCV2 and Mhyo in Argentina

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Introduction

Vaccination against PCV2 and *Mycoplasma hyopneumoniae* (Mhyo) has become an efficient tool to control and reduce economic losses associated with Circovirosis and Enzootic Pneumonia (EP)[1]. The aim of this field trial was to compare the effect on productive parameters of two different vaccination commercial protocols against Mhyo and PCV2 in a commercial farm in Argentina.

Materials and Methods

The field trial was performed in 2019 in a 600 sows farm and 4.565 animals were included. Productive parameters and lung lesions scores were collected from 7 batches (2.248 animals) vaccinated with Circovac® and Hyogen®, Ceva at 3 WOA (G1) and 7 batches (2.317 animals) vaccinated with Vaccine B (PCV2+Mhyo RTU – 2 shots) at 3 and 6 WOA (G2). The economic balance in the 2 groups was calculated using Respinomics®app.

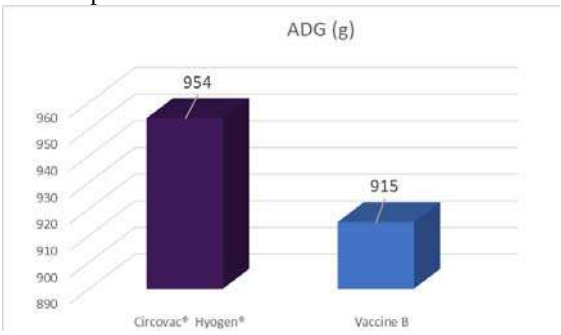
Results

Animals vaccinated with Circovac® and Hyogen® showed 32% of Bronchopneumonic lungs (BP) while animals vaccinated with vaccine B presented 69.5% of BP ($p<0.05$). The EP-Index (calculated from the frequency and severity of EP-like lesions) was on average by 1.1 in G1 and the animals vaccinated with Vaccine B were 4.23 ($p<0.05$). Table 1. Statistical difference was also observed in performance: Circovac® and Hyogen® vaccinated animals had 954 g of ADG (period that corresponds between 70 to 161 days of life) whereas Vaccine B vaccinated animals had 915 g of ADG (Figure 1). From the performance data the financial profit was U$ 1.065 per animal vaccinated with Ceva vaccines.

Table 1. Enzootic pneumonia like lesions summary

	Bronchopenumonic lungs (%)	EP Index
Circovac® Hyogen®	32%	1.1
Vaccine B	69.5%	4.23

Figure 1. ADG (g) in both groups submitted to different vaccination protocols



Conclusions and Discussion

In this field study, the single dose vaccinations with Circovac® and Hyogen® improved pigs' lung health and farm's profitability due to better growth performance. Similar results were also found in other studies comparing vaccine protocols[2,3,4]

Moreover, it was less stressful for the pigs compared to the double shot vaccination.

Thus, Circovac® and Hyogen® are important tools to contribute in reduce the economic impact of these two important pig diseases and improved animal welfare compared to double-dose vaccination.

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Efficacy of combination of Hyogen® and Circovac® vaccines (Ready to mix, RTM) on the control of Mycoplasma and Porcine Circovirus infection under field conditions

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Introduction

The objective of this study was to assess the efficacy of combination of Circovac® and Hyogen® vaccines, ready to mix (RTM), on the control of Mycoplasma and Porcine Circovirus infection under field conditions.

Materials and Methods

A total of 1,295 weaned pigs (at 4 weeks of age) were divided into two groups. Control group (n=700) was vaccinated with a commercial porcine circovirus type 2 (PCV2) and Mycoplasma hyopneumoniae (M. hyo) combination vaccine at 4 weeks of age. RTM Group (n=595) was vaccinated with a combination of Circovac® and Hyogen® (0.5 mL and 2.0 mL), ready to mix (RTM), at 4 weeks of age. Lung lesions scores were measured for enzootic pneumonia-like lesion (EP-like) using B.E.Straw's method [1, 2] at slaughter. Pig performance parameters including mortality rate, average daily gain (ADG), weight at slaughter were recorded and calculated.

Results

Result showed that prevalence of EP-like lesion in RTM group was 72.2%, compared with 79.3% in control group. Average EP-like lesion affected lung surface was 9.3% and 11.7% in RTM and control groups, respectively. In the fattening period, pig mortality rate was significantly reduced in RTM group (2.6% VS. 7.0%, p=0.002). Average daily gain (ADG) in RTM group was significantly higher compared with that of control group (755 g/d VS. 695 g/d, p<0.001). Furthermore, RTM pigs had significantly shorter average days to slaughter compared with control pigs (117.2 days VS. 121.9 days, p<0.001). However, there were no differences in pig performance during nursery period.

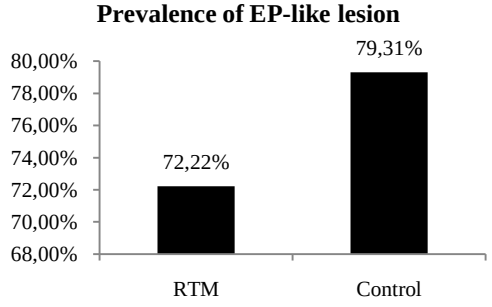

Figure 1. The prevalence of Enzootic pneumonia-like lesion of pigs in RTM and control group.

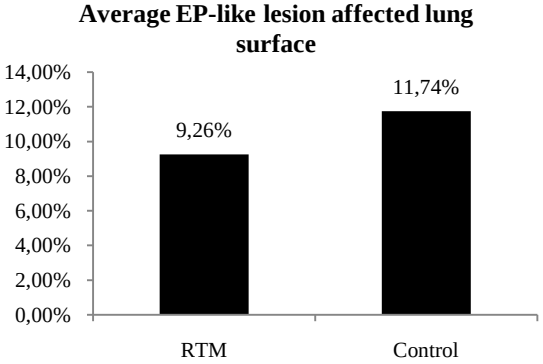

Figure 2. The average Enzootic pneumonia-like affected surface of pigs in RTM and control group.

Table 1. Summary of the performance parameters in fattening period of pigs in RTM and control group.

Parameters	RTM	Control	p-value
Mortality rate (%)	2.61%	7.02%	0.002
ADG (g/d)	755	695	<0.001
Slaughter weight (kg)	106.7	106.0	0.1791
Days to slaughter (d)	117.20	121.91	<0.001

Conclusions and Discussion

In conclusion, the combination of Circovac® and Hyogen® vaccines, ready to mix (RTM), showed better efficiency in growth performance under field conditions. The use of Circovac® and Hyogen® RTM reduces mortality rate and EP-like lesion, and improves growth performance during the fattening phase comparing with conventional PCV2/M. hyo combination vaccine.

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Evaluation of the effect of two different *Mycoplasma hyopneumoniae* vaccines on the weight and homogeneity of carcasses at slaughter

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Introduction

Mycoplasma hyopneumoniae (Mhyo) causes enzootic pneumonia, one of the most important respiratory diseases due to its economic impact in swine production worldwide. These economic losses are associated with reduced growth rate, poor feed conversion ratio and increased medication cost[1]. Moreover, differences in growth rate during growing-fattening period may lead variability of body weight (BW) and, consequently, the weight and value of pig carcasses[2]. Commercial vaccines against *Mhyo* are used for controlling this agent and reducing its impact on production parameters. Therefore, the objective of the present study is to evaluate the effect of two different *Mhyo* commercial vaccines on the weight and homogeneity of carcasses within slaughtered pig batches.

Materials and Methods

Two batches (B1 and B2) were selected from a *Mhyo* positive two-site farm. Piglets were vaccinated at three-weeks-old with vaccine A (n=900, May 2018) in B1 and with Hyogen® (n=900; May 2019) in B2, following the manufacturer's instructions. To minize the potential effect of housing and climatological conditions on the prevalence of *Mhyo* infection, both batches were selected in spring (May), allocated in the same nursery and fattening facilities, and reached the market BW for slaughtering in winter (December). At the end of fattening period, data regarding average daily gain (ADG), feed conversion rate (FRC), mortality rate, medication cost and total production cost per batch (€/kg) during fattening period were collected. Once at slaughter, individual carcass weight was also obtained. Homogeneity on carcass weight between batches was compared using the Levene's Test. Differences between groups were assessed using the Tukey test. Significant differences were considered when *p-value<0.05*.

Results

Performance parameters recorded during fattening period are shown in Table 1. Average of carcass weight from piglets vaccinated with Hyogen® (B2) was higher (88.02±8.01 kg) compared to those pigs vaccinated with vaccine A (79.85±9.27 kg). Distribution of individual carcass weight values for both batches are shown in Figure 1. Weights of pig carcasses from batch B2 also were significantly more homogeneous than in batch B1 ($p<0.05$).

Table 1. Performance parameters recorded from batches B1 and B2 during fattening period.

Performance parameters	Batches		
	B1 (Vaccine A)	B2 (Hyogen®)	B2 – B1*
ADG (g)	717.8	723.0	+ 5.2
FCR	2.595	2.533	- 0.062
Medication cost (€)	1.69	1.39	- 0.30
Mortality (%)	3.27	3.02	- 0.25
Production cost (€)	1.19	1.17	- 0.02

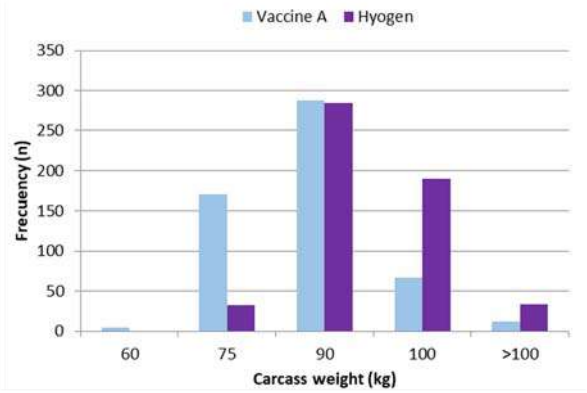

Figure 1. Distribution of individual carcass weights per batches.

Conclusions and Discussion

These results demonstrated piglet vaccination against *Mhyo* with Hyogen® improved all performance parameters evaluated in this study during growing-fattening period. Moreover, pigs vaccinated with Hyogen® also showed higher BW and homogeneity at slaughter, increasing the profit of growing-finishing period. Thus, piglet vaccination against *Mhyo* with Hyogen® leads to improve growth performance parameters and, consequently, weight and quotation of pig carcasses at slaughter.

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Dynamics of pro-inflammatory cytokine gene expression in pigs experimentally infected with *Mycoplasma hyopneumoniae*

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Introduction

Mycoplasma hyopneumoniae is the causative agent of Swine Mycoplasmal Pneumonia (SMP) and is responsible for losses in the pork industry, mainly due to reduced performance of infected animals. Immunopathological events are pointed out as critical in the formation and severity of cranioventral consolidation lesions (1). Several cytokines were shown to be produced at lesion sites using immunohistochemistry (2), but few studies looking at cytokine coding gene's expression and dynamics over time are available. This study aimed to assess the dynamics of cytokine coding gene expression over a 56 days period post infection in pigs experimentally inoculated with *M. hyopneumoniae*.

Materials and Methods

A total of 24 Large White male pigs of 28 days of age and originating from a certified *M. hyopneumoniae*-free farm were used in this study. The animals were randomly allocated in a control group (n=8) and inoculated group (n=16). Animals were intracheally inoculated with either 10 mL of sterilized Friis medium (control animals) or 10 mL of lung inoculum diluted in sterilized Friis medium, containing 10^7 CCU⁄mL of *M. hyopneumoniae* pathogenic strain 232, obtained from Iowa State University (IA-USA) (inoculated group).

Every 14 days, two control and four inoculated animals were randomly chosen and humanely euthanized. Necropsies were performed at 14, 28, 42 and 56 dpi. Macroscopic lung lesion samples were collected, immediately flash frozen in liquid nitrogen and stored at -80° C. Tissue samples were submitted to RNA extraction using RNeasy Blood and Tissue Plus kit (Qiagen, USA). The extracted RNA was converted into cDNA using Superscript IV First Strand Synthesis kit (Thermo Fisher, USA). Specific primers targeting the coding genes of Interleukin-1α (IL-1α), Interleukin-1β (IL-1β), Interleukin-6 (IL-6), Tumor Necrosis Factor- α (TNF-α) were used in qPCR reactions, which were performed in a real time thermocycler CFX 96 (Bio Rad, USA) using Quantitect® Sybr Green master mix (Qiagen, USA). The specificity of amplicons was assessed through dissociation curve, performed at the end of 40 cycles. Normalization of target gene expression was using *rpl-4* gene as reference and according to $2^{-\Delta\Delta Cq}$ (3) calculation. Data analysis and graphs were performed on software GraphPad Prism 6 (La Jolla, CA-USA). Differences were considered significant when $p<0.05$.

Results

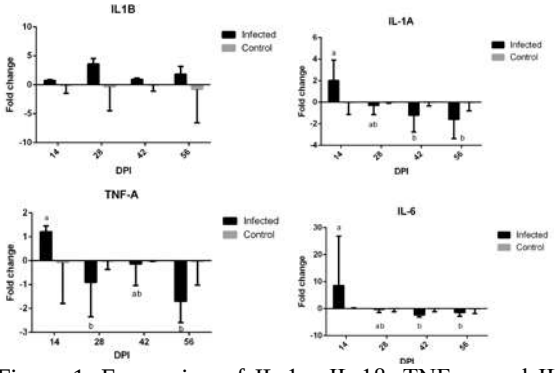

Figure 1. Expression of IL-1α, IL-1β, TNF- α and IL-6 coding genes in lung lesion samples of *M. hyopneumoniae* experimentally infected pigs at 14, 28, 42 and 56 dpi.

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Conclusions and Discussion

IL-6 gene was upregulated only at 14 dpi group reinforcing the idea that this cytokine could be directly related to initial lesion formation (4). The expression of IL-1α and IL-1β genes were both upregulated at 14 and 28 dpi respectively, both cytokines are known to be involved in activation of inflammation at its beginning, resulting in fever, vasodilation response and acute phase proteins production (5). IL-1β was reported as markedly involved in the host response to *M. hyopneumoniae* infection, mainly regarding cellular immunity development and tropism of inflammatory cells to the lesion site (6). A significantly different upregulation of TNF-α gene at 14 dpi indicated that this cytokine, and its effects, are required in the beginning of the inflammation caused by *M. hyopneumoniae* infection, as expected due to its pro-inflammatory role.

Acknowledgments

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Comparison of *Mycoplasma hyopneumoniae* serum antibody assays

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Introduction

Antibody response to *Mycoplasma hyopneumoniae* (*MHP*) is commonly evaluated with serum ELISAs. In the field, serology provides rapid results at an affordable cost. The goal of this study was to compare six commercial *MHP* serum ELISAs using samples from animals of known mycoplasma infection status.

Materials and Methods

Fifty 8-week-old CDCD pigs were allocated to 5 inoculation groups with 10 pigs per group: (1) negative control (2) *M. flocculare (MFLOC*) (3) *M. hyorhinis* (*MHR*) (4) *M. hyosynoviae* (*MHS*), and (5) *MHP*. Serum (2X per week) and oral fluid (daily) samples were collected through 56 days post-inoculation (DPI). Oral fluid and lung tissue from the *MHP* group were tested by PCR to confirm productive infection. Oral fluid samples from non-*MHP* groups were tested to confirm freedom from *MHP* infection.

Six commercial *MHP* serum antibody ELISAs were compared: (1) M hyo ELISA SK 108 BioChek, Borkshire UK (2) INgezim M. hyo COMPAC, ©Eurofins Ingenasa, Madrid, Spain (3) M. hyo Ab Test, IDEXX Laboratories Inc., Westbrook, ME USA (4) ID Screen® *Mycoplasma hyopneumoniae* Indirect, IDvet, Grabels France (5) CIVTEST® SUIS MHYO, Laboratorios HIPRA, S.A., Girona Spain, and (6) IDEIA™ *Mycoplasma hyopneumoniae* EIA kit, ©Oxoid Limited, Hampshire UK. Test comparisons were based on misclassification error rates. ELISAs 2 to 6 had “suspect” categories, but because “suspect” is not a viable category in the field, analyses were performed with “suspect” considered positive and “suspect” considered negative. For the analysis, false positives were defined as any positive from non-*MHP*-groups and false negatives were defined as negative results from *MHP*-inoculated pigs≥ 21 DPI . Notably, no positive results were observed≤ 21 DPI, but no penalty was imposed for two “suspect” results (DPIs 14 and 17). The six ELISAs were then compared by Poisson regression (PROC GENMOD, SAS v.9.4)

Results

A total of 680 serum samples were collected. Analyses of the results from non-*MHP*-inoculated pigs using manufacturers' recommended cut-offs resulted in zero false positives, with the exceptions of ELISAs 4 and 6 (Table 1). False positive responses were seemingly random, i.e., no pattern(s) in cross reactivity with *MFLOC*, *MHR* or *MHS* groups was detected.

Notably, both pigs and ELISAs contributed to the variation in the *MHP* serum antibody response. Among *MHP*-inoculated pigs, "suspects" were sporadically observed as early as 14 DPI (ELISA 6, 2 of 8 pigs), with continuous improvement in antibody detection at DPI ≥ 28.

Table 1. ELISA false positive and cumulative misclassification error rates

ELISA	FPR[1]	FPR[2]	TDP[3]	MER[4]	MER[5]
1[6]	-- 0 --		24	1.86	1.86
2	0	0	28	2.07[7]	2.36[7]
3	0	0	28	1.79	1.79
4	4	5	28	3.28[7]	3.00[7]
5	0	0	24	1.71	1.64
6	1	14	14	1.14	1.86

[1]FPR: False positive rate, with "suspect" results interpreted as negative. [2]FPR: False positive rate, with "suspect" results as positive. [3]TDP: time in days to detect first seropositive in *MHP*-inoculated pigs. [4]MER: misclassification error rate, with suspect as negative. [5]MER: misclassification error rate, with suspect as positive. [6]ELISA 1 has no "suspect" category. [7]Significant difference in MER ($p \leq 0.05$).

Conclusions and Discussion

MHP antibody detection is a useful tool for establishing the status of pig populations. In the field, early detection is paramount, but false positives are highly disruptive to production flows. No significant difference in misclassification rates were detected among ELISAs 1, 3, 5, 6. ELISA 6 achieved the earliest antibody detection, but also produced the most false positives results; ELISAs 1, 3, and 5 produced no false positives, but were slower to detect *MHP* serum antibody. The tug-o-war between diagnostic sensitivity and diagnostic specificity remains a dilemma for *MHP* surveillance and justifies further research on assay improvement.

Conflict of interest

The authors declare no conflicts of interest with the exception that JZ has served as a consultant to IDEXX Laboratories, Inc. on areas of diagnostic medicine independent of this research.

Conserved Influenza virus hemagglutinin peptide adjuvanted with CAF01 induce strong immune response against pandemic H1N1 virus challenge in pigs

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Introduction

Swine is one of the animal models of reference for Influenza Virus (IV) research due to its similarity to humans. Current influenza vaccines differ every year according to the circulating strains due to the mutagenic nature of these viruses. For this reasons, the search for a universal influenza vaccine based on a conserved peptide is one of the main goals in IV research.

Our research group has defined and worked with a well-conserved 34 amino acid peptide (NG34) from hemagglutinin (HA1). The role of this peptide has been demonstrated in eliciting strong cellular and humoral immune response leading to protection against homologous as well as heterologous influenza virus infection in different animals including mice, chickens (1) and pigs (2).

The present study aimed to compare the use of novel adjuvants in combination with NG34 peptide and a current commercial trivalent IV seasonal vaccine.

Material and methods

Thirty pigs were distributed into 5 experimental groups of 6 animals each: Sham-vaccinated/unchallenged; Sham-vaccinated/ challenged; NG34 peptide adjuvanted with CAF01; NG34 with CDA+GalCerMPEG adjuvant; and Seasonal Trivalent Influenza vaccine (STIV). Animals were immunized twice in a time interval of 21 days. After 42 days of the first vaccination, piglets were challenged by intranasal and endotracheal routes with pandemic H1N1 strain A/Catalonia/63/2009. Temperature and clinical signs were monitored throughout the experiment. After challenge, pigs were euthanized at 3 and 7 dpi. Samples were collected on vaccination, challenge and necropsy days. Viral shedding was assessed by RT-qPCR in nasal swabs and BALF. Cell immunity was assayed by IFNγ ELISPOT and lymphocyte subsets by flow cytometry. Serology was measured by ELISA, IHA and seroneutralization assays.

Results

Piglets showed a peak of fever on day 1 after challenge. Influenza gross lung lesions were present in all groups; higher variability and exacerbation was observed in NG34-CDA/ αGalCerMPEG and STIV groups where lesions were more prominent than sham-vaccinated challenged group. Animals vaccinated with NG34-CAF01 reduced the viral load in the upper and lower respiratory tract while rest of the groups did not clear completely the virus. NG34-CAF01 animals induced IFNγ secretion upon stimulation with the peptide. Cytometric analysis revealed different cell subsets among groups. NG34-CAF01 elicited a strong IgG response against the NG34 peptide in sera compared to the NG34-CDA/αGalCerMPEG where no significant antibody response was observed.

Discussion and conclusions

Comparison of different adjuvants and vaccines against IV infection in pigs showed different severity of lung lesions, viral clearance, cell and antibody responses. Peptide NG34 combined with CAF01 adjuvant could represent a potential vaccine candidate against IV infection due to its efficacy in reducing the viral load in lung, nasal shedding and decreasing lung lesions in contrast to other adjuvant combinations or vaccines and thus reduce spread of infection potentially preventing pandemics.

Acknowledgements

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Comparative efficacy evaluation of two C-strain vaccines and an E2 subunit vaccine in CSF maternally derived antibody positive piglets

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Introduction

Classical swine fever (CSF) is an important swine disease in China, and sporadic outbreaks with mild clinical signs were reported in spite of compulsory vaccination. One possible reason for vaccine failure could be inference from maternally derived antibodies at vaccination in the field [1]. The aim of this study is to evaluate the efficacy of two live attenuated C-strain vaccines and an E2 subunit marker vaccine in the presence of maternally derived antibodies (MDAs).

Materials and Methods

Groups of 10 piglets each were vaccinated either intramuscularly with pre-licensing C-strain vaccine serial produced in PK cell line (C-PK) by Boehringer Ingelheim, or commercial C-strain vaccine produced in ST cell line (C-ST) or subunit E2 vaccine (Subunit E2) at the age of 23-25 days. Only piglets vaccinated with C-ST vaccine were boosted 5 weeks later. At 65-day post first vaccination, all vaccination groups together with a challenge control group were received 1mL of highly virulent Shimen virus ($10^{6.0}TCID_{50}$/mL) intramuscularly. Rectal temperatures and clinical score (CS) were recorded daily [2]. The subcuti, tonsil, lymph node, lungs, kidney, bladders, button ulcers in the colon and spleen were examined for CSF lesions at necropsy. The presence of CSFV genome in blood and nasal swab samples at 3,7,10 and 16 days post challenge was determined by real-time RT-PCR..

Table 1. Clinical and virological results after challenge

Group	No of piglets	Fever	Average clinical score[1]	Average pathological lesion[2]	Mortality	ADWG	Feed/ growth	Viremia[3]	Virus shedding[3]
C-PK (1 shot)	10	1/10	0 (0/160)	0.09 (7/80)	0% (0/10)	0.881	2.133	20% (8/40)	5% (2/40)
C-ST (2 shots)	10	2/10	0.26 (42/160)	0.21 (17/80)	0% (0/10)	0.772	2.346	22.5% (9/40)	7.5% (3/40)
Subunit E2 (1 shot)	9	9/9	8.24 (1137/138)	0.35 (25/72)	11% (1/9)	0.208	5.308	65.7% (23/35)	60% (21/35)
Challenge control	5	5/5	15.27 (626/41)	0.88 (35/40)	100% (5/5)	-0.926	-0.347	100% (11/11)	100% (11/11)
Negative control	5	0/5	0 (0/80)	0 (0/40)	0% (0/5)	0.868	1.727	0% (0/20)	0% (0/20)

Note: [1]Average clinical score=Total CS in the group /(No. of piglets*days recorded before died or necropsy). [2]Average pathological lesion= Total gross lesions of the group/(number of piglets*8 tissues observed). [3]Viremia and shedding= Total frequencies detected positive of the group/(number of piglets*4 time points tested)*100%.

Results

In the presence of MDAs, vaccination with a single shot of C-PK or two shots of C-ST vaccine protected against severe clinical signs and significantly reduced viremia and virus shedding. Only a few piglets developed transient viremia and virus shedding. Strikingly, the piglets vaccinated with one shot of Subunit E2 vaccine had severe clinical signs with high level of viremia and virus shedding and one piglet died at after challenge, despite robust E2 antibodies induced. In line with clinical and virological results, piglets vaccinated with one shot of C-PK vaccine had best ADWG and feed conversion ratio among three vaccination groups.

Conclusions and Discussion

The results demonstrated that a single shot of C-PK vaccine is efficacious in the presence of MDAs. The overall performance is superior over two shots of C-ST vaccine routinely applied in the field. The efficacy of single shot Subunit E2 vaccine in MDA positive population is questionable. One limitation of this study is that only small number of animals included in this control experiment, though they were from commercial pig farms. Large field studies will be conducted in the future.

Acknowledgments

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Long-term immunity of a C-strain vaccine against the predominant subgenotype 2.1 strain of classical swine fever virus

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Introduction

In China, classical swine fever has been controlled by mandatory vaccination with C-strain vaccine. Despite this, sporadic outbreaks have been reported [1]. It raised questions about the virulence and pathogenicity of prevalent subgenotype 2.1 strains, and the efficacy of C-strain vaccines against them. To investigate this, the virulence of three field isolates was evaluated in weaned piglets and compared with the highly virulent reference Shimen strain. Duration of immunity (DOI) of a C-strain vaccine against field isolate GD191 was assessed at 15 months post vaccination.

Materials and Methods

In virulence assessment study, animals were aged 6 weeks ± 3 days and were randomly assigned to one of five challenge groups: GD191 (n=10), GD18 (n=10), QZ-07 (n=9), Shimen (n=10) and a control, unchallenged group (n=5). Animals were given an intramuscular injection of 1 mL of 10^5 $TCID_{50}$/mL of virus stock or phosphate-buffered saline (PBS) control.

In DOI study, 10 piglets were randomly assigned to groups 1 and 2. They were vaccinated with $10^{4.5}$ $TCID_{50}$ of C-strain vaccine stock produced by Boehringer Ingelheim or placebo intramuscularly at the age of 3 weeks, and were received 1 mL of GD191 field-strain virus ($10^{5.1}$ $TCID_{50}$/mL) intramuscularly at 15 months post vaccination. On the day of challenge, a further strict control group (group 3) and a challenge control group were included with weaned piglets at three weeks of age.

After challenge, rectal temperatures and clinical score (CS) were recorded daily [2]. The subcuti, tonsil, lymph node, lungs, kidney, bladders, button ulcers in the colon and spleen were examined for CSF lesions at necropsy. The presence of CSFV in blood and nasal swab samples at 3,5,7,10 and 16 days post challenge was determined by standard virus isolation assay.

Results

Clinical signs for the field strains ranged from severe to mild and mortality ranged from 80%–0% (Table 1). In comparison with the highly virulent Shimen strain, GD191 strain has moderate-to-high virulence, GD18 has mild virulence and QZ-07 has low virulence. This data support the use of field strain GD191 as a genotype 2 challenge virus to assess the efficacy of C-strain vaccines.

A robust antibody response was seen in all vaccinated animals and lasted up to 15-months post vaccination. Challenge with GD191 strain, no fever or clinical signs was seen in vaccinated pigs. Only fever, no clinical sign and mortality were observed in unvaccinated, challenged adult pigs. In contrast, fever and severe clinical sign were observed, and three of five piglets died in challenged weaned piglets.

No viremia or nasal shedding was detected in the vaccination. All pigs in the two challenge control groups were at least transiently viraemic. Nasal shedding was seen in three of five adult pigs and in all piglets. Pathological lesions were seen only in the bladder of 2/5 animals in challenged adult pigs. In challenged weaned piglets, lesions were seen in the tonsil, spleen, kidney, lymph nodes and bladder.

Table 1. Virulence assessment of CSF field isolates

Virus	Incubation period	Mean Clinical score	Mean pathological score	Mortality
Shimen	1-2	>15	13	100%
GD191	4-6	>15	11.9	80%
GD18	6-8	5	6.3	10%
QZ07	7-9	1-2	2	0%

Table 2. Summary of DOI study

Group	Fever	Clinical sign	Mortality	Viremia	Virus shed
Vaccination group	0%	0%	0%	0%	0%
Challenge (adult pigs)	40%	0%	0%	100%	60%
Strict control	0%	0%	0%	0%	0%
Challenge (weaned pigs)	80%	100%	60%	100%	100%

Conclusions and Discussion

This study has confirmed that a C-strain vaccine confers sterilizing immunity against the prevalent genotype 2 field strain. In contrast to the historical genotype 1 strain, the recent genotype 2 field strain caused different clinical manifestations in adult and weaned piglets. Adult pigs showed subclinical infection with viral shedding, whereas weaned piglets showed overt signs of infection. Virus shedding to the environment and the transmission of infection from asymptomatic adult pigs to their offspring may account for some of the sporadic outbreaks of CSF.

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Intradermal and subcutaneous administration of polyvalent FMD vaccine induced high levels of homologous neutralizing antibodies persisting until slaughtering in pigs

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Introduction

Intramuscular (IM) FMD vaccination has been widely used in susceptible animals including pigs, but development of granulomatous lesions in muscular compartments has been cited as a critical adverse effect in vaccinated animals (1). Otherwise, while intradermal(ID)/subcutaneous(SC) administration is highlighted as a potential substitutive measure to overcome the incidence of local adverse effect, current vaccine adjuvants and formulation are not compatible with induction of sufficient immune responses in field conditions. The purpose of this study was to determine whether a new polyvalent FMD vaccine, which has incorporated the latest emulsion technology and suitable adjuvants to be used by either ID/SC route, induce high levels of neutralizing antibodies against homologous FMDV strains in conventional pig farms.

Materials and Methods

A total of 146 eight weeks old (wo) pigs from three pig farms were used. Polyvalent single-oil emulsion FMD vaccine (Bioaftogen® ID, Biogénesis Bagó) containing 3 FMDV strains (O_1/Campos, A_{24}/Cruzeiro, A_{2001}/Argentina) was used via two administration routes: 66 intradermally, 65 subcutaneously, and 15 were remained as unvaccinated controls (CON). The first and second vaccination were conducted at 8 wo and 11-12 wo with a dose of 0.5ml, respectively. Bleeding were carried out 7 times: at 0, 14, 28, 42, 56, 84, and 112 days post-vaccination (dpv). Virus neutralizing antibody (VN) titers against each strain were determined, and the 50% end-point VN titers were calculated as the reciprocal of $\log_{10}$ final serum dilution that neutralized 100 $TCID_{50}$ of FMDV. The cut-off levels were set at $\log_{10}$ titers compatible with 75% expected percentage of protection (EPP) which estimates the likelihood that vaccinated animals would be protected against a challenge of 10,000 infective doses: VN titers ≥1.65 for O_1/Campos, ≥1.36 for A_{24}/Cruzeiro, ≥1.43 for A_{2001}/Argentina.

Results

VN titers at 0 dpv were low and did not differ significantly between groups (p>0.05). In vaccinated pigs, average VN titers above the cut-off level were induced at 28 dpv for O1/Campos, and at 14 dpv for A_{24}/Cruzeiro and A_{2001}/Argentina. After the 2nd vaccination, VN titers against all strains increased markedly and persisted until 112 dpv. The results indicated that both ID and SC routes are satisfied to induce effective immunity against vaccine strains.

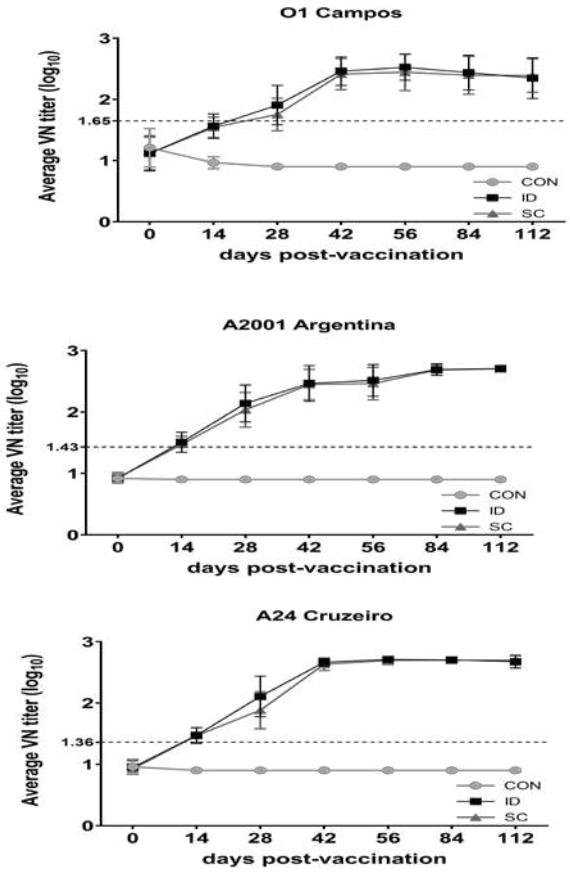

Figure 1. Average VN titers against each vaccine strain. Cut-off level (a dotted line) indicates a titer compatible with 75% EPP, and bars indicate SD.

Conclusions and Discussion

According to a previous laboratory study, ID FMD vaccination induced a very efficient immunological response in pigs (2). Similarly, in the field conditions, Bioaftogen® ID inoculation successfully induced mean VN titers ≥75% EPP which represents clinical protection against homologous FMDV strains, and the protective levels of antibodies persisted until slaughter age. In conclusion, ID/SC route could be a good alternative for IM application in conventional pig farms, inducing efficient immune responses against corresponding FMDVs.

Acknowledgments

This work was supported by CARESIDE Co., Ltd., Republic of Korea.

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Viability of the ADV virus contained in live attenuated vaccines after their combination with inactivated vaccines against Swine Erysipelas and Porcine Parvovirus

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Introduction

Immunization plans are crowded by multiple injections of vaccines. Practices such as the combination of vaccines are sometimes adopted in the field to overcome this problem, however this could jeopardize the safety and efficacy of the vaccines, as inactivated vaccines contain preservatives and other compounds which could compromise the viability of live attenuated vaccines after they are combined. The aim of this study was to evaluate the *in vitro* viability of the Aujeszky's Disease Virus (ADV) contained in a live attenuated vaccine (AUSKIPRA® GN) after it was combined with an inactivated vaccine against *Erysipelothrix rhusiopathiae* (ERYSENG®) and another one containing *E. rhusiopathiae* and porcine *Parvovirus* (ERYSENG® PARVO).

Materials and methods

AUSKIPRA® GN was combined by re-suspending its lyophilized fraction with the commercial vaccines ERYSENG® PARVO and ERYSENG®, whilst the lyophilized fraction of AUSKIPRA® GN from the same batch was re-suspended with the standard solvent (red solvent) provided by the manufacturer and this solution was used as a control. The prepared solutions were then left at room temperature and the viability of the ADV virus contained in them was assessed every hour for 3 hours post preparation (hpp). The viability of the ADV was tested *in vitro* on a culture of PK15 cells and by a Cell Culture Infectious Dose 50 ($CCID_{50}$) assay. The cytopathic effect (CPE) titre was calculated by Kärber.

Results

The batch of the ADV vaccine used for the study was shown to maintain the titres of the live virus within the specifications of the product ($\geq 10^{5.5}$ $DICC_{50}$/dose), after its preparation and up to 3 hpp. Moreover, no major drop in titre was observed after the combination of the vaccines (average ADV titre $CCID_{50}$/dose with ERYSENG® of $10^{6.56}$ and with ERYSENG® PARVO of $10^{6.84}$); compared to the control (average ADV titre $CCID_{50}$/dose of $10^{6.80}$).

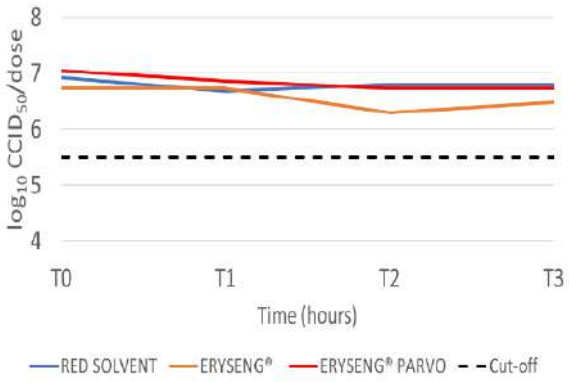

Figure 1. ADV viability. $CCID_{50}$ of ADV after the preparation of the standard ADV vaccine (blue line) or the combination of this with ERYSENG® (orange line) and ERYSENG® PARVO (red line). The slashed line represents the minimum titres of live virus specified by the ADV vaccine manufacturer.

Discussion and conclusion

The results suggest that the non-licensed combination of the studied ADV vaccine (AUSKIPRA® GN) and the inactivated vaccines against *Erysipelothrix rhusiopathiae* (ERYSENG®) and *E. rhusiopathiae* and *Porcine Parvovirus* (ERYSENG® PARVO) does not jeopardize the viability of the live attenuated virus contained in the ADV vaccine. Further studies in animals are needed to evaluate the safety and efficacy of the combination of these products.

Acknowledgments

The authors thank the R+D and UCAM unit staff for their support to the study.

Viability of the PRRS virus contained in a live attenuated vaccine after its combination with an inactivated vaccine against Glässer disease

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Introduction

Immunization plans in sows are already crowded by multiple injections of vaccines. Practices like combination of vaccines are sometimes adopted in field to overcome this problem; when not licensed, this might jeopardize the safety and efficacy of the vaccines. Inactivated vaccines may contain preservatives and other compounds that might compromise the viability of live attenuated vaccines after their combination; as a consequence, the efficacy of this might altered.

This study aims to evaluate the *in vitro* viability of a PRRSv live attenuated vaccine after its combination with an inactivated vaccine against Glässer disease.

Materials and methods

Vaccines were combined by re-suspending the lyophilized fraction of UNISTRAIN® PRRS with HIPRASUIS® GLÄSSER (whereas the dissolvent of UNISTRAIN® PRRS was discarded). Moreover, the lyophilized fraction of a UNISTRAIN® PRRS of the same batch was re-suspended with the dissolvent provided by the manufacturer and this solution was used as control. The prepared solutions were then left at room temperature and the viability of the PRRS virus contained in them was assessed each hour up to 4 hours after preparation. The viability of PRRSv was tested *in vitro* by immunoperoxidase monolayer assay on a culture of MARC-145 cells. Results were expressed as $\log_{10}$ of Cell Culture Infectious Dose 50 ($CCID_{50}$).

Results

The batch of the PRRS vaccine used for the study maintained, after its preparation and up to 4 hours of in-use stability, the titres of the live virus within the specifications of the product ($10^{3.5\text{-}5.5}$ CCID/dose), either combined with its own solvent or the Glässer vaccine. When compared to control at T0, no major decrease of PRRSv titre was observed after the combination of the vaccines. Indeed, the combination of vaccines showed similar average virus titres at each time point evaluated compared to control.

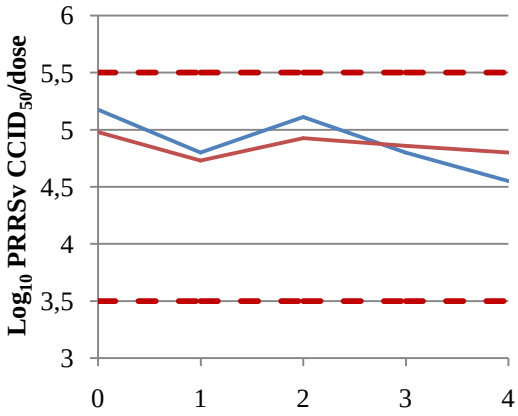

Figure 1. PRRSv viability expressed as $\log_{10}$. $CCID_{50}$ after (a) the preparation of the PRRS vaccine with its own solvent (blue line, control) or (b) the combination of the PRRS vaccine with the Glässer vaccine (orange line, test combination). Red lines represent the minimum and maximum titers of live virus specified by the PRRS vaccine manufacturer.

Discussion

Results suggested that the non-licensed combination of the studied PRRS and Glässer vaccines does not compromise *in vitro* the viability of the live attenuated virus contained in the former. In particular, the viability of PRRSv was maintained during the entire in-use stability of the product after combination of vaccines. Further studies in animals with several vaccine batches are needed to evaluate the safety and efficacy of the combination of these products.

Conclusion

The PRRS virus contained in UNISTRAIN® PRRS remains viable *in vitro* after combination with HIPRASUIS® GLÄSSER up to 4 hours of in-use stability.

Acknowledgments

The authors thank the UCAM unit and the R&D department staff for their support to the study.

Comparative study of the safety of Glässer disease vaccines in Brasil

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Introduction

Glaesserella (Heamophilus) parasuis is a main pathogen affecting pig industry, causing meningitis, polyserositis, polyarthritis and bacterial pneumonia; an infection known as Glässer's disease (1). Antimicrobials have been commonly used to treat this bacterial disease in farm animals, but the emergence of antimicrobial resistance, a serious threat for public health, presses for the implementation of alternatives for disease control (2). Vaccines are the preferred alternatives to control the affection, as they have demonstrated to be useful to prevent this disease (1). Still, a common perception is that when these vaccines are applied to piglets, they may cause reaction in lactation or early nursery stage; a very inconvenient moment as a lack of growth at this stage can be correlated to a reduction in productive parameters (3). The objective of the present study was to determine the safety of the most used commercial vaccines of Brasil against *Haemophilus Parasuis* in postweaning piglets.

Materials and Methods

The trial was performed in a commercial farm of 1500 sows in Patos de Minas (Minas Gerais, Brasil) with Dan Breed genetics. A total of 130, 24-day old piglets were selected for the trial and divided into 4 groups (Control, vaccinated with PBS (n=10); vaccinated with HIPRASUIS® GLÄSSER, HIPRA (n=30); vaccinated with A (multiple antigen vaccine) (n=30); vaccinated with B (vaccine with tocopherol in adjuvant) (n=30). Rectal temperatures were recorded just after vaccination, 4 hours, 1 day and 2 days post-vaccination. Differences between groups were tested through an ANOVA test, and a Post-Hoc Tukey test if ANOVA was significant. The percentage of piglets with fever (assuming fever as temperature higher than 40ºC) was analyzed at 4 and 24 hours post-vaccination. The differences were tested through an ANOVA of a logistic regression and, in case of significant differences, a post-hoc Tukey test was performed. All statistics were performed with R software.

Results

Mean rectal temperatures are shown in table 1 and figure 1. Rectal temperatures at 48h were not statistically different and have not been included. The percentage of animals with fever (>40ºC) at 4h and 24h post vaccination are reported in figure 2.

Table 1. Mean rectal temperatures (ºC).

	Control	HIPRASUIS GLASSER®	Vaccine A	Vaccine B	*P*-value
0 h	39,3	39,52	39,48	39,53	*0,16*
4 h	39,73 (A)	39,68 (A)	40,38 (B)	40,43 (B)	*<0,001*
24 h	39,63	39,6	39,8	39,94	*<0,001*

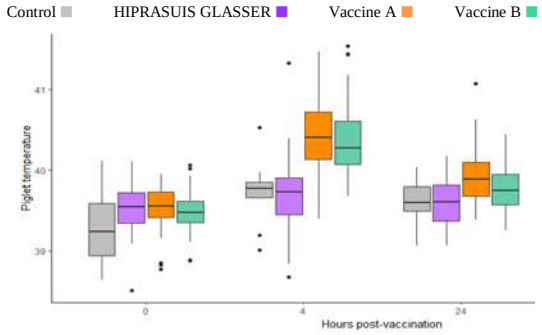

Figure 1. Mean rectal temperatures (ºC).

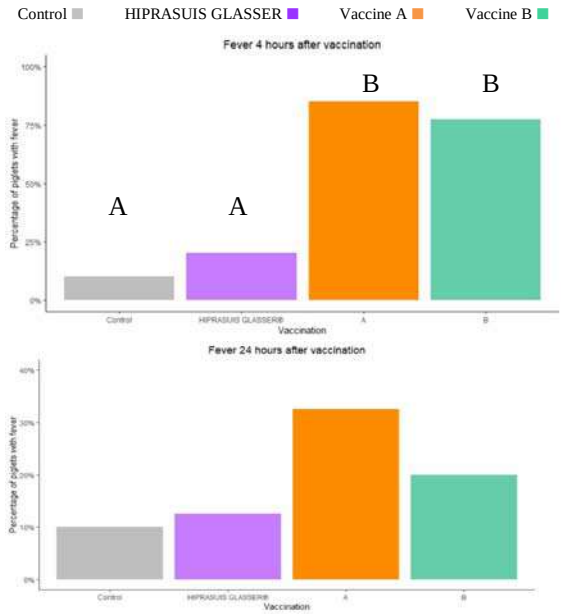

Figure 2. Percentage of animals with fever (>40ºC).

Conclusions

Vaccine A and vaccine B have been reported to cause a significant increment in body temperature from 4 to 24h post-vaccination. Moreover, percentage of animals with fever with these two vaccines was also significantly increased at 4h and numerically at 24h post-vaccination. HIPRASUIS® GLÄSSER is the safest alternative evaluated in the trial, as no significant increment in temperatures was recorded.

Acknowledgments

HIPRASTATS Statistical service for the statistical analysis of the data.

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Comparative study of the safety of Mypravac® Suis and Hiprasuis® Glasser administered on the same side versus administered on different sides of the neck

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Introduction

Vaccination against *Mycoplasma hyopneumoniae* and *Glaesserella (Heamophilus) parasuis* is a common practice in the Philippines. Often these vaccines are given at the same time, during the lactation stage, with minimal or no adverse reactions. In recent years, one-shot mycoplasma vaccines have grown in popularity due to the convenience they offer. However, in terms of efficacy, it is well known that one-shot falls short compared to a two-shot mycoplasma vaccine (1).

The objective of the present study was to determine the safety of the combined administration of 2 monovalent 2-shot vaccines on the same side using a double barrel vaccinator, simulating the use of a bivalent vaccine. This protocol would enable farmers to obtain the efficacy of two two-shot vaccines with easy administration.

Materials and Methods

The trial was performed on a commercial farm of 500 sows in Batangas, Philippines with PIC mixed genetics. A total of sixty 20-day old piglets was selected for the trial and divided into 3 groups (piglets vaccinated with HIPRASUIS® GLÄSSER and MYPRAVAC® SUIS on the same side of the neck using a double barrel vaccinator (n=25), were named the "Combined" group; those vaccinated with HIPRASUIS® GLÄSSER and MYPRAVAC® SUIS on different sides of the neck (n=25), were the "Separate" group; and those vaccinated with PBS (n=10), were the "Control" group. Rectal temperatures were recorded just after vaccination and 1 day and 2 days post-vaccination. Vaccination sites were observed, and any significant change was noted. Differences between groups were tested by an ANOVA test, and a Post-Hoc Tukey test if ANOVA was significant. All statistics were performed with R software.

Results

No significant reactions were observed in any of the animals after vaccination (data not shown).

Mean rectal temperatures are shown in figure 1 (1st shot) and figure 2 (2nd shot).

No animals were reported to have fever (>40ºC). Temperatures remained consistently within a physiologic range and were not increased with either combined or separate administration compared to the PBS control in any of the observations. A tendency to increase (P=0.1) was observed only in the group that received a separate application in comparison to the control and combined groups on the day of the second vaccination (39.01ºC vs 38.6ºC and 38.6ºC respectively). Nevertheless, temperatures always remained within the normal range and no local reactions were observed.

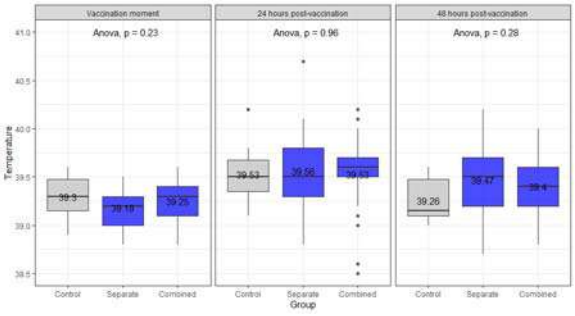

Figure 1. Mean rectal temperatures at 1st shot (ºC).

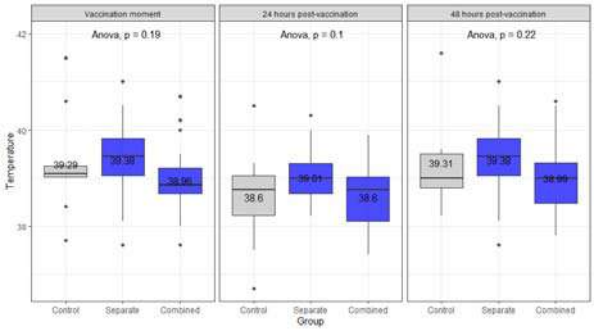

Figure 2. Mean rectal temperatures at 2nd shot (ºC).

Conclusions

The combined administration of HIPRASUIS® GLÄSSER and MYPRAVAC® SUIS on the same side of the neck using a double barrel vaccinator is a practical and safe procedure that can reduce farmers' workload.

Acknowledgments

HIPRASTATS Statistical service for the statistical analysis of the data.

References

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Efficacy of VEPURED against Edema Disease in Thai Pig Farming

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Introduction

Porcine Edema Disease (ED), caused by *E. coli* that produces F-18 pili and verotoxin 2e (VT2e), is known to cause critical losses in the period shortly after weaning (1). Moreover, against the background of the reduction of antibiotic use, the pig industry is facing calls for alternatives to antimicrobials to control the disease.

In this study we aimed to evaluate the field efficacy and safety of VEPURED®, a new recombinant vaccine against pig ED.

Materials and Methods

This study was conducted on a commercial farrow-to-finish farm with 1,800 sows during March 2019. At the beginning of the year, this farm experienced a negative impact on productivity and economic losses due to ED. The disease was diagnosed by detecting the gene encoding VT2e (VEROCHECK, HIPRA) together with clinical symptoms and high mortality and culling losses of pigs (14.06%).

For the trial, a total of 18,503 piglets were randomly allocated into two groups. Briefly, Group 1 piglets (n = 9,409) received 1 dose (1 ml) intramuscularly administered at 2 to 8 days of age in accordance with the manufacturer's instructions. Group 2 (n = 9,094) was the control group (unvaccinated piglets). The efficacy of VEPURED® under field conditions during the nursery and finisher stages was compared between groups using T-test statistical analysis (IBM SPSS statistics base 22.0). The return on investment (ROI) after immunization during the finisher period was calculated as formerly noted (2).

Results and Discussion

Table 1 and Table 2 include productive parameters.

Pig performance was improved during the nursery and finishing periods; with a reduction in the mortality rate of up to 24.23%. In addition, group 1 (vaccinated pigs) tended had a significantly higher average daily gain (ADG) than group 2. This improvement in farm performance during the grower-finisher period was calculated to have a ROI of 604% (based on historic pork price circumstances up to 2.35€/kg).

The current study demonstrates that VEPURED® is safe and effectively provides protection against *E. coli* infection causing Edema disease in pigs.

Table 1. Nursery period productive parameters.

Productive parameter	VEPURED®	Control	*P*-value
No. batches	10	10	-
No. piglets	9,409	9,094	-
% Mortality	0.64[a]	3.07[b]	0.002
ADG (g/day)	351.42[a]	322.80[b]	0.01
FCR	1.53[a]	1.49[a]	0.11
Exit age (days)	65.58[a]	65.37[a]	0.50
Exit weight	21.58[a]	20.03[b]	0.01
Cost of exit kg	2.57 €	2.71 €	-

[1]a,b superscripts indicate statistically significant differences within the main effect ($p \leq 0.05$)

Table 2. Finishing period productive parameters.

Production parameter	VEPURED®	Control	*P*-value
No. batches	5	11	-
No. piglets	4,662	7,472	-
% Mortality	3.26[a]	4.05[a]	0.38
ADG (g/day)	618.54[a]	595.86[b]	0.04
FCR	2.57	2.57	0.95
Exit age (weeks)	24.99[a]	24.90[a]	0.68
Exit weight	100.65[a]	97.13[b]	0.04
Cost of exit kg	1.28 €	1.29 €	-

[1]a,b superscripts indicate statistically significant differences within the main effect ($p \leq 0.05$)

Acknowledgements

The authors would like to thank the commercial swine farm and veterinarians in Nong Bua Lamphu province, Thailand co-operatives for providing oral fluid samples, retrospective production parameters and their encouragement.

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Comparison of cell viability in fresh and frozen porcine colostrum

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Introduction

The ingestion of colostrum by piglet in the first hours of life is crucial for its health status, since the transfer of maternal immunity occurs passively through colostrum (3). Colostrum contains lymphocytes (T and B cells), phagocytes (neutrophils and macrophages), and epithelial cells (1). Colostrum can be storage at 4°C or freezing, however, the freezing and thawing process can damage the cells (2). As little is known about the cell viability and immunogenicity of colostrum, this study aimed to evaluate fresh and frozen porcine colostrum and the effect on immune cells viability.

Materials and Methods

Colostrum was manually collected from 20 sows (multiparous and primiparous) after farrowing and was diluted in PBS containing 5% fetal calf serum. Then, it was centrifuged and the fat upper layer was discarded. Colostrum evaluation was carried out in fresh and in frozen colostrum after 7 and 15 days of storage at -20°C. The viability of cells was evaluated by trypan blue exclusion test. The flow cytometry was performed with Accuri® flow cytometer (Becton Dickinson) and 50.000 events were analyzed. For this, colostrum samples were incubated with the following antibodies: isotypes controls; mouse anti-pig CD3, CD4, CD8, granulocytes, macrophages, CD27, CD45RA, CD79a, CD5, CD14, CD16, IgM, CD45RA/B220, and CD335. The data were pre analyzed by the K-S test. The Friedmann test was used for longitudinal analyses, with the Student-Newman-Keuls posttest, whereas transversal analyses were performed using the Kruskal-Wallis test, with the Student-Newman-Keuls posttest.

Results

Table 1 shows the evolution of granulocyte, NK cells ($CD3^-CD8^{low}CD335^+$), $macrophages^+$, monocyte/ macrophage ($macrophages^+CD14^+CD16^+$), lymphocytes ($CD3^+$, $CD5^+$, $CD79a^+IgM^+$, $CD79a^+$ $CD45R/B220^+$, $CD5^+$) percentage in fresh and frozen colostrum after 7 and 15-days period. The colostrum freezing resulted in a significant decrease of $CD4^+$ $CD3^+$ T cells and its subsets, such as naive $CD4^+$ T cells ($CD4^+CD27^+CD45RA^+$), central memory $CD4^+$T ($CD4^+CD27^+CD45RA^-$), and effector memory $CD4^+$T cells ($CD4^+CD27^-CD45RA^-$). Besides, $CD8^+$ $CD3^+$ T cells and a subset of central memory $CD8^+$T cells ($CD8^+CD27^+CD45RA^+$) were also decreased with colostrum freezing. Moreover, B-lymphocyte ($CD79a^+$) showed a decrease after freezing, but B-lymphocyte subsets did not differ. No variation was observed on the immune cells ($p>0.05$) after frozen for 7 up to 15 days, although the number of them were decreased after 15 days at -20°C.

Table 1. Comparison of cell viability in fresh and frozen porcine colostrum.

Immune cells	Storage conditions		
	Fresh	Frozen for 7 days	Frozen for 15 days
GRANULOCYTES	9.282±1.549	7.140±1.215	8.645±1.890
MACROPHAGES$^+$	40.61±1.265	40.56±1.142	37.42±1.356
CD14$^+$CD16$^+$	14.15±1.441	13.20±0.878	10.80±1.000
CD335$^+$	14.93±1.117	14.39±1.549	15.85±1.850
CD79a$^+$	15.00±1.807^a	5.384±0.937^b	6.132±1.485^b
CD19$^+$, IGM$^+$	22.65±2.372	20.18±3.314	19.53±1.970
CD19$^+$, CD45R/B220$^+$	3.399±0.366	2.406 ±0.834	1.974±0.556
CD5$^+$	38.45±1285^a	34.93±0.972^b	32.15±1.225^b
CD3$^+$CD4$^+$	10.72±1.535^a	6.121±1.542^{ab}	5.219±1.251^b
CD4$^+$CD27$^+$CD45RA$^-$	13.90±2.132^a	10.53±1.091^a	4.835±1.415^b
CD4$^+$CD27$^+$CD4RA$^+$	4.790±0.861^b	3.944±0.634^b	1.94±1.999^a
CD4$^+$CD27$^-$CD45RA$^-$	25.77±2.265^a	22.50±2.002^a	9.575±1.534^b
CD3$^+$CD8$^+$	7.580±0.954^a	4.190±0.982^b	3.976±0.895^b
CD8$^+$CD27$^+$CD45RA$^-$	14.66±1.772	11.32±1.138	9.893±1,339
CD8$^+$CD27$^+$CD45RA$^+$	5.836±1.278	4.468±0.647	5.394±1.229
CD8$^+$CD27$^-$CD45RA$^-$	22.10±1.977^a	20.30±1.948^{ab}	14.96±1.591^b

$^{a, b}$ $p<0.05$ *vs other treatment.*

Conclusions and Discussion

Taking into account only the stability of the immunological components studied, it seems reasonable to recommend freezing storage for 15 days for −20°C. Under this condition, the contents of almost all the bioactive factors evaluated were maintained, except for subsets of T-lymphocytes and B-lymphocytes cells. A longstanding porcine colostrum storage study is underway. The understanding of the effect of storage on immune cells in colostrum is of great importance, mainly for the acquisition of passive immunity by the newborn piglet fed with frozen colostrum.

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Comparison of immune cells of colostrum from gilts and sows

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Introduction

The early and sufficient intake of good quality colostrum is essential for piglet's health and growth. After farrowing, the newborn piglet goes from a sterile intrauterine environment to an antigen-rich external environment, which requires an adequate immune response to survive (1,2). In swine, maternal immunity is transferred to offspring only after birth via colostrum ingestion. In addition, porcine mammary secretions contain leukocytes, which are absorbed by the neonate and produce a measurable immune activity and numerous soluble factors with antimicrobial and/or immunomodulating activity (2). This study aimed to compare the immune cells presented in colostrum from gilts and sows.

Materials and Methods

Eighty dams (40 gilts and 40 sows) had the farrow induced by an analog of prostaglandin F2a (Alfabédyl®) on day 113 of gestation and colostrum was manually collected after the birth of the first piglet. Teats were scrubbed with alcohol and iodine and gloves were worn to minimize contamination. Colostrum was diluted in PBS containing 5% fetal calf serum, centrifuged and the fat upper layer was discarded. The cells' viability was evaluated by the trypan blue exclusion test. For flow cytometry, colostrum samples were incubated with the following antibodies: isotypes controls; mouse anti-pig CD3, CD4, CD8, granulocytes, macrophages, CD27, CD45RA, CD79a, CD5, CD14, CD16, IgM, CD45RA/B220, and CD335. Flow cytometry was performed with Accuri® flow cytometer (Becton Dickinson) and 50.000 events were analyzed. The data were pre analyzed by the K-S test. The transversal analyses were performed using the Kruskal-Wallis test, with the Student-Newman-Keuls posttest.

Results

The dams' colostrum was composed of 30% of lymphocytes, 40% of macrophages, and neutrophils are the predominant granulocyte in mammary secretions (Table 1). It was observed a numerically but not a statistically significant increase in granulocyte, NK cells ($CD3^-CD8^{low}CD335^+$) and B-lymphocytes subsets in sows compared with gilts. Sows showed $CD4^+$ T cell subsets and monocyte/macrophage ($macrophages^+CD14^+CD16^+$) significantly higher than gilts ($p \leq 0.05$). The phenotypic classi fication of $CD4^+$ T cell subsets that increased are naive $CD4^+$ T cells as $CD4^+CD27^+CD45RA^+$, central memory $CD4^+$T cells as $CD4^+CD27^+CD45RA^-$, effector memory $CD4^+$T cells as $CD4^+CD27^-CD45RA^-$, and central memory $CD8^+$T cells as $CD8^+CD27^+CD45RA$.

Table 1. Comparison of immune cells of colostrum from gilts and sows.

Immune Cells	Gilts	Sows
$Granulocytes^+$	7.062±1.589	11.06±2.408
$Macrophages^+$	40.74±1.050	40.51±2.180
$CD79A^+$	16.47±3.064	13.83±2,216
IGM^+	18.93±1.085	25.63±4.022
$CD45R/B220^+$	1.856±0.547	2.846±0.470
$CD5^+$	32.36±1.820	32.53±1.884
$CD3^+CD4^+$	9.354±2.277	11.81±2.117
$CD4^+CD27^+CD45RA^-$	8.380±1.215[a]	18.31±3.110
$CD4^+CD27^+CD4RA^+$	2.955±0.761[a]	6.258±1.274
$CD4'CD27^-CD45RA^-$	21.53±2.360	29.16±3.331
$CD3^+CD8^+$	7.205±1.536	7.880±1.266
$CD8^+CD27^+CD45RA^-$	10.09±1.706[a]	18.33±2.348
$CD8^+CD27^+CD45RA^+$	3.848±1.077	7.426±2.050
$CD3^-CD8^{low}CD335^+$	13.20±1.098	16.31±1.735
$Macrophages^+CD16^+$	27.60±1.286	25.90±1.945
$Macrophages^+CD14^+CD16^+$	10.88±1.695[a]	16.76±1.888

[a]Superscripts indicate statistically significant differences within main effect ($p \leq 0.05$)

Conclusions and Discussion

Dams' colostrum contains lymphocytes (T and B cells), phagocytes (neutrophils and macrophages), and epithelial cells. The estimated number and types of specific cells in colostrum vary widely between dams and are affected by parity order. This study provides new data about population of cells existing in porcine colostrum, mainly regarding to subsets of T-lymphocytes and the difference about these subsets in gilts and sows' colostrum.

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Administration of Progressis to boost previous PRRSV immunity in sows increases transfer of maternally-derived antibodies and contribute to decrease PRRSV incidence

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Introduction

In PRRS endemically infected farms, the establishment of optimized immunization protocols for gilts and sows is critical to reduce the impact of the disease. Commonly, the immunization programs start by applying MLV vaccines and these are then used again as recall antigens every three or four months throughout the productive life of the sow. While this schedule is adequate to maintain immunity in sows, the transfer of maternally-derived antibodies to the offspring is relatively scarce. Strategies aimed to increase the duration and amount of antibodies may help to decrease the reproduction rate in nurseries or to delay the peak of the incidence. The aim of the present study was to test the concept of priming with an MLV and boosting with Progressis® PRRS (CEVA, santé animale, France) in order to control PRRSV infection in farrowing units and in nurseries of PRRSV positive unstable farms.

Materials and Methods

Two PRRS-endemic farms were selected for the study. The first one was a 300-sow farm run on a three-week batch system. The second was a 1,700-sow farm with weekly farrowing batches. In both cases, farms were unstable in spite of the application of a vaccination program including a primo-vaccination with a MLV and recall doses with the same vaccine at least three times per year. Nurseries were highly infected, with incidences closer to 100% by the end of that phase. The design of the experiment established two groups of sows, both primo-vaccinated with a MLV vaccine at 60 days of gestation. One group was re-vaccinated at 90 days of gestation using Progressis® (PG group), while the other one, non-vaccinated at this time, and was the Control (C group). Furthermore, for the PG group, gilts were vaccinated additionally with Progressis® after the initial primo-vaccination in the acclimation (before mating) of the farm. The offspring in both groups was followed from birth (umbilical cord sampling) to the end of the nursery period (bleeding at 2, 4, 6 and 9-10 weeks of age) to assess viral circulation (RT-qPCR) and the levels of antibodies (ELISA).

Results

At the beginning of the study, PRRSV was detected at weaning, confirming that the farms were unstable. For farm 1, six batches of parturitions were examined, accounting for 155 sows (77 PG and 78 C) with 785 piglets followed (389 from PG sows and 396 from C sows). When the aggregated results were examined, the proportion of viremic piglets at 6 weeks of age was higher in the offspring of C sows (15.5%) compared to the offspring of PG sows (8.8%, p=0.03), resulting in a relative risk of 1.38. Similarly, the cumulative incidence from birth to 6 weeks of age tended to be lower in the offspring of PG sows (15.1 vs. 23.7%, p=0.07). The proportion of piglets with anti-PRRS antibodies at weaning was also significantly higher for PG piglets (94.6% vs 83.9%, p<0.001).

In farm #2, the offspring of 155 sows was followed (77 PG and 78 C), totaling 866 piglets (448 PG and 418 C). In this farm, the cumulative incidences between samplings at 3, 6, and 9 weeks of age were not significantly different. These results were affected by an explosive circulation of the virus at 6 weeks of the third batch (>97% incidence for both groups). If considered the third batch as an outlier, in the two first batches the cumulative incidence at 6 weeks of age was significantly lower in PG group (11.3% vs 22.5% of incidence; p=0.03), resulting in a relative risk of 1.8. At weaning, the proportion of piglets with detectable anti-PRRS antibodies was higher for the PG group (p=0.02).

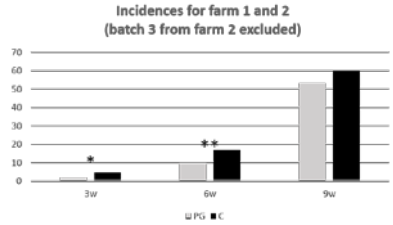

Figure 1. Aggregated incidences for farm 1 and 2 (third batch from farm 2 excluded). PRRSV incidence was significantly lower at 3 and 6 weeks of age for PG group. *p<0.05. **p<0.01.

Conclusions and Discussion

Administration of Progressis® to sows before farrowing was efficacious to modify the dynamics of the infection in piglets, as shown by the lower proportion of infected pigs at 3 and 6 weeks of age. The most likely explanation of these results is the higher proportion of animals with maternally-derived antibodies at weaning. The use of Progressis® to boost humoral responses and to enhance colostral-derived protection is a promising and safe approach that can help unstable farms where conventional MLV-alone strategies are not sufficient.

Acknowledgments

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Impact of different vaccine protocols against porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* on piglet performance from weaning to slaughter and lung lesions score

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Introduction

The availability of safety and effective vaccines against porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* (Mh) exists on the market, but there are differences in their composition, especially in adjuvants. Some adjuvants, especially oily ones, cause more intense adverse reactions in animals, which although transient, have a direct and negative impacts on average daily weight gain[1].

The aim of this study was to measure the impact of adverse reactions using different vaccines for PCV2 and *Mycoplasma hyopneumoniae* (reactive vs nonreactive) on piglets from weaning to slaughter and also the extension of lung lesions (Mh like lung lesions).

Materials and Methods

The study was conducted in a large cooperative in northwest of Rio Grande do Sul state – Brazil. 870 piglets were divided in 2 groups: Treatment 1 (T1): piglets were vaccinated with Ingelvac Circoflex® + Ingelvac Mycoflex® (FLEXcombo® - Boehringer Ingelheim Vetmedica Inc.), single dose of 2 mL injected intramuscularly at 28 days of age and Treatment 2 (T2): piglets were vaccinated with Circumvent PCVM® (MSD Animal Health) 2 doses of 2 mL at 28 and 42 days of age.

At weaning, 28 days of age (D0), all animals were individually identified with numbered earrings with the same color and randomized by weight. With 62 days of age (D34), piglets were weighed again and transferred to a finishing barn. With 169 days of age (D141), animals were weighed individually to obtain the final weight. At slaughter (179 days of age), 269 lungs were evaluated (134 from T1 and 135 from T2) according to Straw[5]. The performance data were submitted to the T- test.

Results and Discussion

Zootechnical performance and lung lesions area (%) are shown in **Table 1**. The piglets performance immunized with FLEXcombo® (T1) was 710 grams superior at D34 when compared with the group that received Circumvent PCVM® (T2) (p = 0.02). In the finish phase, the T1 maintained its superiority in the final weight with a difference of 840 g between treatments (p=0.09). The worst performance of T2 animals probably was due to the adverse reaction caused, mainly after the second dose of the vaccine, which contains oil as the adjuvant. These side effects are characterized by swelling at the application site, lethargy and hyperthermia of up to 2°C, which cause a decrease in feed intake for up to 2 days after vaccination. The better performance of T1 piglets probably is due to the use of a vaccine containing a polymer as the adjuvant, which does not cause adverse reactions, and feed intake is maintained right after vaccination[4]. The lung lesions area were low in both treatments, so that, the difference observed between the treatments was irrelevant and not enough to cause delay in the development of animals in T1 (4,76%) when compared with T2 (2,74%). So, the difference observed in lung lesions did not impact the final weight at slaughter, as described by other researchers[1,2].

Table 1. Body weight (Kg) and lung lesions area (%) of piglets submitted to different vaccination protocols (PCV2 and *Mycoplasma hyopneumoniae*).

Parameters	Treatments	
	T1: CIRCOFLEX + MYCOFLEX (FLEXcombo)	T2: CIRCUMVENT PCVM
Body weight (D0)	7,77[a]	7,76[a]
Body weight (D34)	21,61[a]	20,91[b]
Body weight (D141)	105,60[a]	104,76[b]
Lung lesions area (%)	4,76[a]	2,74[b]

ab Means with different superscripts within a row differ significantly (T -test, P≤0.10).

Conclusions

The use of Ingelvac Circoflex® and Ingelvac Mycoflex® (FLEXcombo®) was the best option, because in addition to its efficacy, practicality and safety, it does not cause the impact of adverse reactions on productive performance.

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Impact of different vaccine protocols of *Mycoplasma hyopneumoniae* on performance and lung lesions in swine

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Introduction

Currently, there are several *Mycoplasma hyopneumoniae* vaccines available on the market, with single dose or two dose protocols, depending on the technology of their respective adjuvants. However, due to recurrent respiratory problems and numerous risk factors, some farms have been using adapted vaccination protocols "*off label*", with booster doses of *Mycoplasma hyopneumoniae* (Mh) vaccine throughout the animal's life, with the expectation that this action is the key point for solving the respiratory problems. The objective of the study was to evaluate the impact on average daily weight gain and the extent of lung lesions at slaughter of different vaccination protocols against *Mycoplasma hyopneumoniae*.

Materials and Methods

The study was carried out in a cooperative located in the western region of Santa Catarina - Brazil, with a history of respiratory problems in the finishing phase caused by *Mycoplasma hyopneumoniae*. Eight hundred and sixty six piglets were distributed in 2 different vaccination protocols with Ingelvac Mycoflex® (Boehringer Ingelheim Vetmedica Inc.): **Protocol 1 (P1)** single intramuscular dose of 1 ml at 21 days of age, and **Protocol 2 (P2)** protocol *"off label"* of Ingelvac Mycoflex®, with a booster dose in the housing in the finishing phase (2 doses of 1 ml intramuscularly, at 21 and 63 days of age).

All animals were identified with numbered ear tags, randomized by weight at 63 days of age. At 105 days after housing in the finishing phase (168 days of life), the pigs were weighed again and then slaughtered. At slaughter, 330 lungs were evaluated (157 from P1 and 173 from P2) according to Straw's methodology (1985)[1]. The data obtained were submitted to the t-test.

Results and Discussion

Productive performance and lung lesion area (%) are shown in **Table 1**. The weight of the animals at 168 days of life and the average daily weight gain (ADWG) of the finishing phase did not show significant differences between the two protocols. There was no improvement in the performance of piglets submitted to protocol *off label* with booster with a second dose of the Ingelvac Mycoflex® (P2) when compared to the group that received a single dose of Mycoflex (P1) ($p>0.05$). These results are in agreement with other studies comparing different Mh vaccines (single dose vs. two doses)[2].

The lung lesions area (hepatization lesions suggestive of Mh) were statistically equal in two protocols ($p> 0.05$). Different vaccination protocols against Mh, comparing the Ingelvac Mycoflex® with commercial vaccines of two doses, did not show differences in relation to the score of lung lesions, histopathological lesions and molecular results[3]. The main factor that determines the higher prevalence and severity of lung lesions caused by Mh at slaughter is the higher prevalence of piglets weaned positive for the agent[4]. The sow is the main source of infection for these piglets during the lactation, and actions that aim to reduce this spread, such as vaccination of the sows before farrowing to Mh, have been shown to be effective in reducing lung lesions at slaughter and in controlling the enzootic pneumonia[5].

Table 1. Finishing productive performance (Weight - Kg and average daily weight gain – ADWG in g/day) and lung lesions area (%) of pigs submitted to different Ingelvac Mycoflex® vaccination protocols.

Parameters	Mycoflex® Protocols	
	P1: Single Dose (21 d)	P2: Two doses (21 - 63 d)
Initial weight (63d)	24,75 [a]	24,75 [a]
Finishing weight (168d)	120,58 [a]	120,49 [a]
Finishing ADWG (63-168d)	0,917 [a]	0,916 [a]
Lung lesions área (%)	3,66 [a]	3,98 [a]

* Averages followed by distinct letters in the lines differ significantly by t-test ($p<0.05$).

Conclusions

The use of a booster dose (*off label*) of Mycoflex did not demonstrate productive and health performance advantage in a herd with *Mycoplasma hyopneumoniae* challenge.

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Impact of reactive vaccine against porcine circovirus type 2 (PCV2) in the gilts performance from weaning to selection

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Introduction

The choice of a vaccine is very important for an efficient strategy against porcine circovirus type 2 (PCV2). Some vaccines, especially those containing oily adjuvants, cause more severe adverse reactions (swelling at the site of application, hyperthermia up to 2 ° C, lethargy, reduction of feed intake), which can directly reduce daily weight gain[1,2]. This study measure the impact of adverse reactions caused by reactive vaccine against PCV2 on gilts weight gain from weaning until selection.

Materials and Methods

The study was conducted in a farrow to finish herd with 2650 sows from a large company in Rio Grande do Sul State, Brazil. Fifty-four gilts were monitoring from one day after weaning (29 days of age) until the selection (150 days of age). One day after weaning (D0) each gilt was individually identified with numbered ear tag, weighed and randomized by weight in 2 groups with different PCV2 vaccination protocol. Group 1 **(G1)** received a single dose of 1 mL of Ingelvac Circoflex® (Boehringer Ingelheim Vetmedica Inc.) injected intramuscularly on D0 and Group 2 **(G2)** received 2 doses of 2 mL of Circumvent PCV® (MSD Animal Health) with 14 days interval between doses (D0 and D14). Both groups were vaccinated with the same vaccine against *Mycoplasma hyopneumoniae*. At 70 (D41) and 150 days of age (D121), gilts were weighed again individually. At 150 days, gilts were selected according to genetics standards, and the performance data were submitted to the T-test.

Results

There were no clinical cases or mortality caused by circovirus in any of the groups. The performance results are demonstrate in **Table 1**. The weight at 70 days was numerically better in G1 than G2 (28,73 Kg *vs* 27,63 Kg, $p = 0.28$). At 150 days of age, the weight of G1 was 4.4 kg higher when compared to G2 (92,63 Kg *vs* 88,19 Kg, $p < 0.05$). The G1 average daily weight gain (ADWG) was 42 g better in the finish phase ($p < 0.05$), which result in a better cull rate by performance during the selection (G1: 7% *vs* G2: 22%). The G2 had three times more gilts culled due to low ADWG when compared to G1.

Table 1. Body weight (BW - Kg) and Average daily weight gain (ADWG – g/day) of gilts vaccinated with Ingelvac Circoflex® and Circumvent PCV® from one day after weaning until selection (29-150 days).

Productive Performance	PCV2 Vaccine Protocol	
	G1: CIRCOFLEX	G2: CIRCUMVENT PCV
BW (29 d)	6,38	6,36
BW (70 d)	28,73	27,63
BW (150 d)	92,63[a]	88,19[b]
ADWG 29-70d	532	507
ADWG 70-150d	799[a]	757[b]
ADWG 29-150d	707[a]	671[b]

ab Means with different superscripts within a row differ significantly (T - test, $P \leq 0.05$).

Conclusions and Discussion

The worst performance in G2 gilts was probably due to the adverse reaction caused by the oily adjuvant in vaccine used in this group. Lethargy and reduced feed consumption in these animals were observed, characterizing the condition of transient narcolepsy, especially after the second dose. The results obtained are consistent with other field studies showing that reactive vaccines against PCV2 have a negative impact on pig growth[2,3].This study demonstrated that Ingelvac Circoflex® is safe, effective and provides better performance for gilts due the lack of side effects.

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Comparative safety study of different PCV2 vaccines in Thailand

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Background & Objectives

This is part of a larger study to examine differences in efficacy and safety across PCV2 vaccines available in Thailand. Needle free vaccination eliminates needles and is known to reduce stress on piglets(1) and reduces disease risk transmission(3). Reducing the antigen and adjuvant quantities administered, while producing the same immune response(2), may also benefit piglet welfare and feed consumption post vaccination. This study describes the body temperature response following PCV2 vaccination of pigs either intradermally with a needle-free IDAL injector and Porcilis® PCV ID or conventionally with a needle-syringe.

Materials & Methods

A total of 3987 piglets, 28 days of age were distributed in to 4 groups : i) vaccinated with Porcilis® PCV ID intradermally and needle free with IDAL (IDAL); ii) vaccinated with Porcilis® PCV intramuscularly (IM); iii) vaccinated with Circovac® intramuscularly (IM); iv) vaccinated with Pro-Vac® Circomaster intramuscularly (IM); At the time of vaccination, all pigs were picked up by their hind legs and vaccinated according to the label requirements. 20 piglets per group were selected for measurements of body temperature at 0h, 3h, 8h and 24h post vaccination, using an infrared thermometer and taking the body temperature of the chest.

Results

For all groups, peak body temperature was observed at 3h post vaccination. All groups returned to the baseline body temperature by 24h post vaccination. Results were analysed using one-way ANOVA analysis followed by Tukey Test, with cutoff of $p<0.01$ being deemed as statistically significant. All results are summarized in Table 1.

Conclusion

Both intramuscular and intradermal vaccination were able to induce a clear and detectable temperature increase. Pigs vaccinated via intradermal vaccination route returned to normal body temperature baselines the fastest. The present data supports the hypothesis that intradermal vaccination is safe and beneficial for piglet welfare and feed consumption.

Table 1. Average body temperature post vaccination (n=20, ^{o}C).

Time	Porcilis® PCV ID	Porcilis® PCV	Circovac®	Pro-Vac® Circomaster
0h	36.0	36.0	35.9	35.9
3h	36.2^{a}	36.9^{b}	36.9^{b}	36.8^{b}
8h	36.1^{b}	36.6^{a}	36.2^{b}	36.3^{b}
24h	36.0	36.1	36.1	36.0

*$^{a, b}$: values with different superscripts within a row are statistically significantly different ($p<0.01$).

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Field efficacy trial of a novel inactivated injectable vaccine against Lawsonia intracellularis (Porcilis® Ileitis) in Cebu, Philippines

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Introduction

Lawsonia intracellularis is an obligate intracellular bacterium that is the etiologic agent of ileitis in pigs[1,2]. It causes thickened mucosal lining primarily of the small intestine due to infected crypt cell proliferation. Clinical symptoms of ileitis include stunted growth and diarrhea in young, growing pigs whereas hemorrhagic diarrhea and sometimes sudden death in adult pigs. Because of this, the disease continues to be a problem in pig production and is responsible for substantial economic losses worldwide[3,4]. Vaccination and use of antibiotics have been the top control options for ileitis[4]. In this study, the efficacy and safety of a novel inactivated injectable vaccine against *L. intracellularis* (Porcilis® Ileitis) were evaluated.

Materials and Methods

A total of three thousand two-hundred ten (3210) 24-day-old piglets were used in this study. These animals were assigned randomly to one of the two groups: TREATMENT (n=1605), which were vaccinated with Porcilis® Ileitis vaccine 2ml intramuscularly (IM) and CONTROL (n=1605), unvaccinated.

For the trial, serial serum samples were collected as the pigs age. Samples were tested for *L. intracellularis* antibodies with blocking ELISA using Ileitis-ELISA kit in accordance to the manufacturer's instructions (Svanova Biotech AB, Sweden). For the safety trial, production indices were acquired throughout the production cycle.

Results

In Figure 1, both control and treatment groups were negative for *L. intracellularis* at 4 weeks of age. Only in their 10th week when positives started to develop in the control group, whereas the treatment group has been maintaining a positive reading for *L. intracellularis* antibodies as early as 6 weeks up until 18 weeks of age, which highly suggests prolonged protective titers. Furthermore, Table 1 illustrates the observed statistical difference between treatment and control groups based on their production indices. Within assumptions under local conditions, the treatment group calculates a positive economic impact with regards to prime pigs sold, carcass sales, and FCR benefit with a net benefit gain of PhP 499.8 (USD 9.61) per pig compared to the control group.

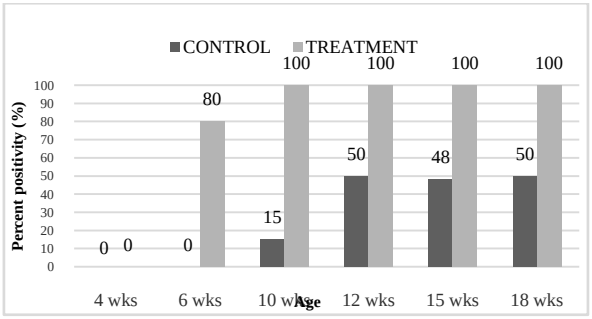

Figure 1. Percent positivity of pigs (%) to *Lawsonia intracellularis* antibodies as they age.

Table 1. Production indices of fattener pigs (T=treatment, C=control groups). a, b: value with different superscripts in each column represent statistically significant differences ($p<0.05$).

	ADG	Birth weight (kg)	Weaning weight (kg)	Transfer weight (kg)	Carcass weight (kg)	Market weight (kg)
T	0.75[a]	1.44[a]	7.47[a]	33.37[a]	117.37[a]	139.14[a]
C	0.74[a]	1.43[a]	7.45[a]	34.05[b]	116.15[b]	137.98[b]

Conclusions

Porcilis® Ileitis demonstrated efficacy in terms of maintaining persistent protective titers, and safety in commercial herds with significant differences over the control pigs for some parameters (transfer, carcass, and market weights) with positive economic effect. As a new, innovative product, Porcilis® Ileitis is a viable option for producers to combat ileitis-related diseases.

Acknowledgement

MSD Animal Health wishes to thank the staff of the farm involved for participating in the study.

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Possibility assessment to emergency vaccine and efficacy of maternally derived antibodies for an attenuated live marker classical swine fever vaccine (Flc-LOM-B^{erns})

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Introduction

Classical swine fever virus (CSFV) is highly contagious and fatal disease of swine (*Sus Scrofa*), affecting domestic pigs and wild boars (1). CSFV is a member of the genus Pestivirus, which belongs to the *Flaviviridae* family. This genome, a single-stranded and positive-polarity RNA, is made up of a sequence of approximately 12,300 nucleotides. CSFV can be further divided into three different genotypes, which in turn are comprised of three or four subgroups. DIVA vaccine, which is capable of antibody distinguishing between vaccine strain and virulence strain in recent years, is of great interest [2]. We developed a CSF live marker vaccine (Flc-LOM-BE^{rns}) with functions as a DIVA. Lactogenic transfer of maternally derived antibodies (MDAs) from immune sows is considered as an effective and economic way to provide passive protection of their piglets from CSFV infection from birth to acquisition of active immunity [3]. In general, the advantage of live vaccine is that they can use as emergency vaccine and maintain the immunity for a long time with one shot, unlike killed vaccine and subunit vaccine. The aim of this study is to investigate whether MDAs from sows immunized with the Flc-LOM-BE^{rns} vaccine can effectively protect their suckling piglets against virulent CSFV challenge. It was also to investigate of the Flc-LOM-Berns vaccine as an emergency vaccine and as long immune sustained vaccine.

Materials and Methods

1) Virulent CSFV infection for pigs with maternally derived antibodies (MDAs): Three groups of 40-60 days piglets, with or without MDAs of Flc-LOM BE^{rns} vaccine, were challenged with virulent CSFV strain. CSFV-specific antibodies, viremia and pathological changes were monitored during experiment period.

2) Virulent CSFV infection for pigs with active immunity for Flc-LOM BE^{rns} vaccine: Three groups of 60 days piglets, immunized with Flc-LOM BE^{rns} vaccine, were challenged with virulent CSFV strain at each 3, 7, and 14 days of post-vaccination.. CSFV-specific antibodies, viremia and pathological changes were monitored during experiment period.

3) Maintenances period of the CSF antibody titer for active immunity: Six pigs (60-day-old) were inoculated with the Flc-LOM BErns vaccine and observed for 250 days the neutralization antibody retention period of the Flc-LOM BErns vaccinated pigs.

Results

1) Efficacy of maternally derived antibodies for Flc-LOM BE^{rns} vaccine : Ten pigs (30-50 days old) with maternally derived antibodies (MDAs) from sows with the Flc-LOM BE^{rns} vaccine inoculation was challenged with virulent CSFV and the MDAs groups with the Flc-LOM BE^{rns} vaccine showed half lethal ratio at 5 $\log_2$ maternal immunity antibody titer.

2) Possibility of Flc-LOM BE^{rns} vaccine as an emergency vaccine and Maintenances period of the CSF antibody titer for active immunity: Pigs (60-day-old, no CSF antibody) were inoculated to virulent CSFV at each 3, 7, and 14 days of post-vaccination (Flc-LOM BE^{rns}) (dpv) and three groups challenged showed 0% (3 dpv), 75% (7 dpv), and 100% (14 dpv) survival ratios. Active immunization for the Flc-LOM BE^{rns} vaccine lasted up to 250 days after vaccination.

Conclusions and Discussion

There is little date on how much maternally derived antibodies (MDAs) titer can be protected the pigs against virulent CSFV. These results suggested that MDAs from the sow immunized with Flc-LOM- BE^{rns} vaccine are able to confer full passive immunity to newborn piglets and the Flc-LOM- BE^{rns} vaccine can be used as emergency vaccine after 14 days of post-vaccination maintaining the immunity until slaughter.

Acknowledgments

The Animal and Plant Quarantine Agency and Ministry of Agriculture Food and Rural Affairs, Republic of Kore.

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Comparative efficacy of inactivated and modified-live Porcine Reproductive and Respiratory Syndrome (PRRS) virus vaccines against a heterologous virus challenge

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Introduction

Although modified live virus (**MLV**) vaccines against PRRS virus can be effective, there is abundant evidence that they readily and frequently revert to virulent. Thus, their use constitutes a major safety concern. Alternatively, inactivated virus vaccines are absolutely safe; but they are generally regarded as being ineffective. To examine this notion, we performed a direct comparison of the efficacy of a prototype PRRS inactivated whole virus (**IWV**) vaccine to that of a commercial MLV vaccine against a heterologous virulent virus challenge.

Materials and Methods

Weanling pigs (23 ± 2 days of age) were immunized intramuscularly with either a prototype IWV vaccine (10^8 $TCID_{50}$ per dose before inactivation) mixed with water-in-oil-in-water (W/O/W) adjuvant or with a commercial MLV vaccine (Prevacent PRRS, Elanco). A third group received vaccine diluent mixed with the same W/O/W adjuvant. The viruses in both vaccines, and the challenge virus, belong to the North American PRRS virus genotype. Pigs immunized with the inactivated virus or the mock vaccine received a booster shot 25 days later. Forty days after the first immunization, all of the pigs were challenged intranasally with the virulent PRRS virus strain 16244B which is heterologous (≤96% homology in GP5) to either vaccine. The pigs were monitored for 12 days after the challenge for clinical signs of pneumonia by pulse oximetry, and blood samples were collected to determine the extent of viremia. Thirteen days after the challenge, the animals were euthanized and their lungs scored for the percent of gross lung lesions by a pathologist blinded to the animal treatment. Objective assessment of pneumonia was done by measuring the density of dissected lung lobes by the water displacement method. The extent of viremia was determined by $TCID_{50}$ assay using a pig alveolar macrophage cell line (ZMAC). The frequency of PRRS virus-specific interferon (IFN)-γ-secreting cells (SC) in blood mononuclear cells was determined by ELIspot. Data was analyzed by *t* test (Prism 8.0.2).

Results

Pigs vaccinated with either vaccine stimulated the generation of PRRS virus-specific antibodies in serum and IFN-γ-SC in their blood. As compared to unvaccinated challenged control pigs, both groups of vaccinated pigs exhibited substantial levels of protective immunity, as evidenced by a significantly lessened extent of gross lung pathology (P<0.001), a close to normal lung density (P<0.01), a significantly higher level (P<0.001) of peripheral capillary oxygen saturation (SpO_2), and improved weight gain (P =0.05 for IWV and <0.01 for MLV). Moreover, while all of the mock-vaccinated pigs exhibited high levels of viremia 12 days after the virus challenge, five of the eight pigs immunized with the inactivated virus were no longer viremic at this time, and all of the pigs immunized with the MLV had ceased to be viremic 10 days after the challenge (Fig. 1).

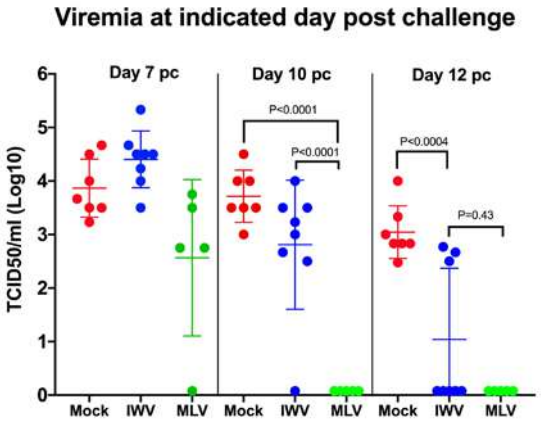

Figure 1. Each symbol represents the level of viremia detected in the serum from a single pig, which are grouped by their treatment. The mean±SD and the P values determined by *t* test are indicated.

Conclusions and Discussion

Although both the IWV and MLV vaccines were efficacious, as indicated by the amelioration of clinical signs, improved growth, curtailing of viremia, and reduced pneumonia, the inactivated vaccine was less efficacious. Nevertheless, all of the parameters measured indicate that a PRRS IWV vaccine formulated with an adequate dose of viral antigen is capable of providing significant levels of protective immunity against a heterologous virus challenge. The results of this study combined with those reported by other investigators are sufficient to oust the notion that inactivated PRRS virus vaccines are ineffective.

Acknowledgment

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Intrauterine growth restriction modifies the proinflammatory cytokine of small intestine in swine during postnatal development

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Introduction

Intrauterine growth restriction (IUGR) compromises fetal development, resulting in low birth weight individuals (1). Some evidences suggest an association between IUGR and increased proinflammatory intestinal cytokines in early life, but little is known about its long-term effects (2). Small intestine cytokines can control proinflammatory mechanisms that result in clinical sequelae such as diarrhea and fibrosis; therefore, variations in the expression of certain cytokine genes can control the response to a given intestinal insult (3). As pigs naturally exhibit severe IUGR, which makes them adequate experimental models, the objective of this research was to investigate the effect of IUGR on the growth performance, evaluating the kinetic expression profile of the proinflammatory cytokines IL-6, IL-1, IL-8, TNF, MCP1 and TLR4 in the duodenum of newborn, weaned, 70-day and 150-day old pigs.

Materials and Methods

Three hundred male piglets were selected and allocated into two experimental groups according to birth weight: high weight (HW) (1.6 - 1.9 kg; n = 150) and IUGR (0.7 - 1.0 kg, n = 150). All animals were weighed at birth, weaning (24 days), 70 days and 150 days of age, and 10 pairs of littermate piglets (10 HW and 10 IUGR) were randomly selected and euthanized for tissue collection. For gene expression studies, mRNA from duodenum samples were preserved in RNA later and the relative expression of genes IL-6, IL-1, IL-8, TNF, MCP1 and TLR4 was quantified by Real Time quantitative PCR. Least squares means were compared using the Student's t-test, with $P < 0.05$ being considered significant. The relative expression of genes was analyzed using the Relative Expression Software Tool (4).

Results

IUGR piglets presented compromised growth performance during all stages of development, as they showed lower body weights and daily gains compared to their HW counterparts (Figure 1; $P<0.01$). During postnatal development (from birth to 150 days of age), IUGR pigs showed upregulation of IL-6 expression, while the upregulation of IL-1 occurred only at younger ages. On the other hand, it was observed that IL-8 and TLR-4 were downregulated in IUGR pigs at 150 days old. These results reveal the strong difference in the expression pattern of proinflammatory markers between both experimental groups, as 150-d old IUGR animals showed high expression of an important inflammatory factor (IL-6) in the enterocytes of the small intestine (Table 1).

Discussion and Conclusion

Gut homeostasis is a regulated process requiring finely tuned complex interactions between growth factors and / or Cytokines (3). Taken together, the results obtained in the present study provide evidence that growth restriction in utero leads to depleted body development from birth to adulthood. In addition, it may permanently affect the proinflammatory cytokine profile, which was shown by the upregulation of IL-6 in particular. It is well known that the increased expression of this cytokine is associated with increased intestinal permeability, which can contribute to the translocation of pathogenic bacteria, besides acting as an inflammation promoter. These findings suggest that IUGR alters the homeostasis of the small intestine due to the high expression of IL-6 from birth to adulthood, which may be involved with the maintenance of permanent inflammation in the intestinal mucosa. In this context, absorption capacity may be compromised in IUGR animals, leading to compromised postnatal growth performance.

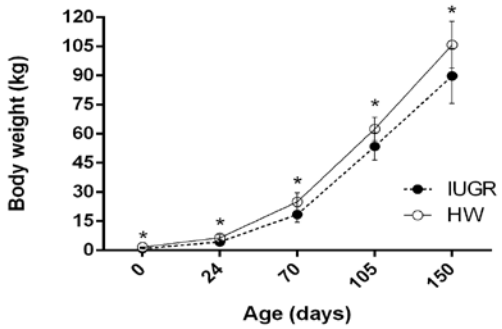

Figure 1: Growth curve from birth up to 150 days of age.* Means statistically different ($P<0.001$).

Table 1 Relative expression of proinflammatory genes from IUGR boars at birth, 24, 70 and 150 days of age

Gene	Birth	P	24d	P	70d	P	150d	P
IL-6	34.5	<.05	0.02	<.05	23.8	<.05	430.5	<.05
IL-1	257.0	<.05	1.65	NS	32.6	NS	1.00	NS
IL-8	0.2	<.05	1.20	NS	0.86	NS	0,15	<.05
TNFa	2.5	NS	0.20	NS	1.64	NS	0.35	NS
MCP1	1.4	NS	0.96	NS	4.36	NS	0.30	NS
TLR4	1.9	NS	0.20	NS	1.08	NS	0.18	<.05

Note: P value < 0.05 describes different relative gene expression. The relative gene expression values are presented in relation to their high birth weight (HW) counterparts. NS: not significant.

Acknowledgments

FAPEMIG,CAPES, CNPq

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Circo/MycoGard® field efficacy assessment after a dual PCV2d/PRRSV174 seeder pig challenge

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Introduction

Circo/MycoGard® is the PCV2/Mhp combination vaccine from Pharmgate Animal Health. In this study, we assessed the efficacy of Circo/MycoGard® after a dual PCV2d/PRRSV174 seeder pig challenge that mimics field infections.

Materials and Methods

A 3,200-head sow farm known to be Mhp, PEDV and PRRSV wild-type negative (MLV vaccinated) sourced piglets for this trial. PCV2 was confirmed stable after PCR negative processing fluids testing. Within each litter, pigs were weighed, ranked by weight, ear tagged and randomly assigned to one of 3 vaccine treatments: Circo/MycoGard® (1mL), CircoFLEX®/MycoFLEX® (2mL) and Saline (1mL). PCV2 vaccines were administered at enrollment in the sow farm prior to weaning as a single dose. Seeder pigs were not vaccinated for PCV2/Mhp. PRRSV MLV (2mL) vaccine was administered to all study pigs at processing time (~3 days of age). Pigs were weaned in a 2-room WTF commercial barn. In each pen, 8 pigs/treatment were randomly allocated plus 3 seeder pigs for a total of 27 pigs/pen. Challenge materials (culture supernatants) for both PCV2d (2mL IN and 1mL IM, 4 $TCID_{50}$/mL) and PRRSV174 (2mL IM, 3.5 $TCID_{50}$/mL) were administered to seeder pigs only at 34 days post weaning/vaccination. Challenge was confirmed by necropsy, histopathology, IHC and PCR detection of both pathogens in treatment contact pigs at 3 weeks post challenge. Mortality and removals were recorded daily and pigs were weighed again at the end of the study (~20 weeks post weaning/vaccination). WTF mortality, removals, ADG, full value and cull pigs were the outcomes for the vaccine efficacy assessment. Data was analyzed using generalized linear mixed models in SAS statistical software.

Results

The non-PCV2 vaccinated treatment group (Saline) performed significantly worse than both vaccinated groups confirming that the seeder pig challenge model was successful and mimicked most field conditions. Both vaccinated groups performed similarly in WTF mortality, removals, ADG, full value and cull pigs as summarized in Table 1.

Table 1 Circo/MycoGard® field efficacy results after a dual PCV2d/PRRSV174 seeder pig challenge.

Parameter	Non-vaccinated	Circo/MycoGard®	CircoFLEX®/MycoFLEX®	p-value
Number of pigs	639	640	641	-
Weaning weight, lb	12.80	12.77	12.79	0.97
Final weight, lb	244.33[b]	255.85[a]	256.71[a]	<0.0001
WTF ADG, lb/day	1.62[b]	1.70[a]	1.71[a]	<0.0001
Full value pigs, %	68.94[b]	88.91[a]	90.65[a]	<0.0001
Dead/Removal, %	25.20[b]	10.16[a]	7.8[a]	<0.0001
Pre-challenge, %	8.57	6.67	3.40	0.50
Post-challenge, %	16.46[b]	4.92[a]	4.18[a]	<0.0001
Cull defect, %	0.29	0.13	0.42	0.08
Cull light, %	3.49[b]	0.37[a]	0.56[a]	<0.0001

Conclusions and discussion

Our results confirmed that Circo/MycoGard® is efficacious and performed similarly to CircoFLEX®/MycoFLEX® in the field after a successful dual PCV2d/PRRSV174 seeder pig challenge, which represented most field scenarios.

Circo/MycoGard® field efficacy after a PCV2d/PRRSV174 individual pig challenge

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Introduction

Circo/MycoGard® is a PCV2b/Mhp combination vaccine with high safety, antigen purity and cell-mediated immunity due to the triple adjuvant system (StimGard® Plus) that it contains. In this study, we assessed the efficacy of Circo/MycoGard® after a dual PCV2d/PRRSV174 individual pig challenge and field conditions.

Materials and Methods

A total of 288 pigs (96 pigs/trt) were assigned to 3 treatments. Circo/MycoGard® (1 mL), Circumvent® PCV-M G2 (2 mL) or saline (1 mL) was administered once at weaning. Treatments were randomly allocated by litter and weight at weaning.

The sow farm was PRRSV, PEDV and Mhp negative. This source was PCV2 positive and recently repopulated (all sows were P2). Pigs were housed in a commercial wean-to-finish (W-F) facility at SSUI Inc. in MN. Each pen contained all treatment groups allocated equally and randomly. Due to finishing space considerations, half of the pigs were randomly selected at challenge for removal at 10 weeks post vaccination (wpv).

Challenge was administered to each pig at 4 wpv. PCV2d was inoculated both IN (2 mL, 1 mL/nostril) and IM (1 mL). PRRSV174 was inoculated IM (2 mL). Processing fluid was collected from the selected farrowing group. Serum was collected from all pigs at weaning and challenge. Tissues were collected from 3 dead/euthanized pigs to confirm challenge at ~2 weeks post challenge.

Performance analysis for W-F (0-20 wpv) was adjusted by weaning weight. Finishing space constraints required removal of half the pigs, so W-F livability was averaged over the two phases and W-F ADG was estimated from the pigs that remained in the study (48 pigs/trt). Primary market pigs (>200lb) and light culls (<200lb) were estimated based on the number of pigs alive at the end of the study.

Results

Both vaccines were equivalent in finishing and nursery performance. W-F livability and ADG were equivalent for both vaccines as detailed in Table 1. Primary market pigs and light culls were equivalent too. Final weight distributions were also equivalent. Non-vaccinated pigs had lighter weights, more variation and underperformed vaccinated pigs.

Processing fluids (n=2) from the selected farrowing group tested PCV2 PCR negative. Serum samples tested 5% (15/288) and 22% (64/288) PCV2 PCR positive at weaning and challenge respectively. ORF2 sequences (n=2) were classified as PCV2a at weaning and PCV2e at challenge. Challenge was confirmed by PRRSV and PCV2 lesions, PCR and IHC in tissues at ~2 weeks post challenge.

Conclusions and discussion

Both vaccines were equivalent in nursery, finishing and wean-to-finish performance under a strong PCV2d/PRRS174 individual pig challenge. Non-vaccinated pigs underperformed vaccinated ones.

Table 1. Circo/MycoGard® efficacy after a dual PCV2d/PRRSV174 seeder pig challenge.

Parameter	Non-vaccinated	Circo/MycoGard®	Circumvent® PCV-M G2	p-value
Nursery pigs, n	96	96	96	-
Nursery Livability (%)	84[b]	88[a]	90[a]	0.01
Nursery ADG (lb/day)	1.00[b]	1.07[a]	1.03[a]	0.02
Finishing pigs, n	49	48	48	-
Finishing Livability (%)	98	100	98	-
Finishing ADG (lb/day)	2.10[b]	2.20[a]	2.19[a]	0.02
W-F Livability (%)	83[b]	88[a]	89[a]	0.01
W-F ADG (lb/day)	1.55[b]	1.65[a]	1.61[a]	0.03
Primary Market pigs (%)	88	96	96	0.21
Light Culls (%)	12	4	4	0.21

Case Report: Benefits of freshly mixed 3-way vaccination on growth performance and vaccination operation during wean to finish

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Introduction

Since ASFV was first reported in China in 2018, both the pig farm management and biosecurity measures have been adjusted for better disease control. One of the key elements of preventing ASFV spread into or inside the farm is to reduce unnecessary operations in the farm, avoiding direct and indirect contact among the animals. Injections are considered one of the risky operations that can spread the disease to other pigs; therefore, more vaccinations increase risk of transmission. Using combined vaccines to minimize the risk of disease spreading while maintaining the production performance in the farm is a suitable option for the current endemic situation. The objective of this study is to monitor the efficacy of a one shot vaccine mixture of PRRS + PCV2 + M.hyo (3FLEX™) compared to vaccination by separate injections in different combination in a production setting.

Materials and Methods

Four farms in China from Jiangxi, Heilongjiang, Liaoning and Henan started the trial on Nov-Dec 2019 and sent pigs to slaughter in April – May 2020. The average day in house was around 150 days.

The piglets of each farm were divided into 2 groups with different tag colors. Group 1, piglets were vaccinated with 3FLEX™ around 3 weeks of age. Group 2, piglets were vaccinated with the current vaccination program in the farm (Farm A and Farm B: Ingelvac PRRS MLV and FLEXCombo™ at the age of day 14. Farm C: Ingelvac® PRRS MLV and local PCV2 vaccine from HARVAC at 14 days of age, MycoFLEX® at 21 days of age. Farm D: Ingelvac PRRS® MLV at 21 days of age, local PCV2 from JINYU and M.hyo vaccine at 10 days of age).

Within each farm, groups were kept under the same environment and management; i.e., room, feed, medication and staff. Slaughter weight, average daily gain (ADG) and mortality rate were the main study parameters.

Results

Pigs were weighed as groups without individual data, due to the biosecurity protocols that did not allow any equipment or visitors go into the pig farms. Therefore, no statistical analysis could be performed in this study.

In Farm A and Farm B, there were no differences in slaughter weight, ADG and mortality between Group 1 and Group 2. In Farm C, slaughter weight and ADG were 1.49kg and 10g better in Group 1 compared to Group 2. In Farm D, slaughter weight and ADG were 3.5kg and 26g better in Group 1 compared to Group 2. Mortality rate of Group 1 was 5% lower (3.2% vs 8.2%) in farm C and 5.19% (0% vs 5.19%) lower in Farm D.

Table 1. Production performance in 4 farms (Gr.1 3FLEX™ vaccinated group and Gr.2 current vaccination program in that pig farm

Parameter	Pig no.	Avg. wean weight (kg)	Avg. Sell weight (kg)	ADG (g/day)	Mortality (%)
Farm A	Gr. 2 PRRS + FLEXCombo™ 14 d				
Gr.1	100	6.56	128.66	842.07	1.0 %
Gr. 2	200	6.60	128.58	841.59	0.5 %
Farm B	Gr. 2 PRRS + FLEXCombo™ 14 d				
Gr.1	105	7.40	100.39	673.82	1.9%
Gr.2	103	7.45	101.15	679.01	2.9%
Farm C	Gr. 2 PRRS 14 d + Local PCV2 14 d + MycoFLEX® 21 d				
Gr.1	95	6.92	119.00	687.61	3.2%
Gr.2	97	7.06	117.51	677.58	8.2%
Farm D	Gr.2 PRRS 14d + Local PCV2 and M.hyo 10d				
Gr.1	75	7.50	130.50	732.14	0%
Gr.2	77	7.35	126.00	706.25	5.19%

Conclusions and Discussion

PCV2 vaccine can reduce viremia, improve weight gain and decrease mortality, but the improvement between different PCV2 vaccines varies.[1] While the study design does not allow statistical comparison the results are consistent with other studies[2,3] . Comparing with vaccination separately with a local PCV2 vaccine, using the 3 way combination results in higher ADG and lower mortality. This will bring better economic returns for the farm, especially under the current situation in which average pig price is around 30 CNY/kg(4.23USD/kg). Meanwhile, these data suggest that 3FLEX™ provides the same protection as separate injections of FLEXCombo™ and Ingelvac PRRS® MLV. Therefore, a 3-way freshly mixed combination can be a better alternative since it minimizes disease spreading with less stress for the piglet by reducing the number of injections, and farms can benefit by saving labor.

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MISCELLANEOUS

Isolation and identification of *Candida* spp. associated with genitourinary infection in sows

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Introduction

Candida spp. emerged as the main pathogens of a variety of infections in humans. The *Candida* genus consists of yeasts that are in the microbiota of men and animals, without causing damage to the host. However, in cases of dysbiosis or immunosuppression, these microorganisms tend to become pathogenic and present aggressive clinical manifestations. Reports that indicate the importance of these agents in pig farming are scarce. In recent years, with the advance of immunosuppressive diseases in the swine herds, such as post-weaning multisystemic wasting syndrome, these fungi may become increasingly important for maintaining the sanitary level and profitability in herds (1). Thus, the present study aimed to isolate and identify the *Candida* species that can play an important role in genitourinary infection.

Materials and Methods

A total of 330 sows were evaluated; 199 vulvar discharge swabs from the deep region of the vaginal canal performed with the aid of speculum sterile and disposable vaginal speculum, and 131 kidney, urinary vesicle and uterus swabs of dead sows. These originated from two herds of Paraná and Mato Grosso states. For isolation, the samples were plated in Sabouraud Dextrose Agar with chloramphenicol (0.1 g/L) and CHROMagar™ *Candida* and incubated in aerobiosis for 72h at 35°C. The isolated colonies were submitted to identification by Matrix Associated Laser Desorption-Ionization – Time of Flight (MALDI-TOF) mass spectrometry. For protein extraction Cassagne et al. (2) and Sendid et al. (3) protocols were followed with some adaptations regarding the centrifugation and incubation time, stirring, formic acid and acetonitrile volumes. The obtained protein spectra were compared to the manufacturer's library using the MALDI BioTyper™ 3.0 software (Bruker Daltonik), and the standard Bruker interpretative criteria were applied.

Results

Sixty-one animals were positive for *Candida* isolation.
From the 84 isolates obtained, 11 *Candida* species were identified of which *C. parapsilosis* (21.4%), *C. krusei* (19%), *C. tropicalis* (15.5%) and *C. catenulata* (15.5%) were the most frequent (Figure 1). Regarding the isolation sites, 83.3% of the isolates originated from the genital system (58.3% from the cervix and 25% from the uterus). The other 16.7% were isolated from the urinary system (8.3 % from the urinary bladder, 6.0 % kidneys, 2.4 % urine). It was observed a greater diversity of *Candida* for the genital system, while only six species were isolated from the urinary system. In 14 animals were isolated from more than one *Candida* species.

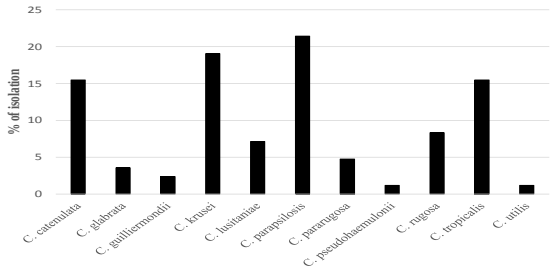

Figure 1. Frequency of *Candida* spp. isolation.

Discussion and Conclusions

Candida is a commensal of the digestive tract. The swine herds can be affected by three important reservoirs of contamination: the pig itself, birds and men (4). Several species of *Candida* cause septicemic symptoms (5), among them *C. tropicalis*, *C. parapsilosis*, *C. krusei* and *C. catenulata*, which presented higher isolation frequency in this work. Several factors such as severe illness or immunosuppression, use of broad spectrum antibiotics, and empirical use of antimycotic medications are factors that may be associated with this infection. Candidiasis in pigs can manifest when host defense is decreased, and was initially reported in piglets fed on food waste and kept in poor sanitary conditions (6). The disease was also reported in pigs from intensive and technified systems in Rio Grande do Sul (Brazil) that were primarily affected by viral diseases (7, 1). The isolation of *Candida* spp. from the genitourinary tract in sows indicates the importance of these agents in production systems, in especially species that may have zoonotic characteristics. The results show a high frequency of isolation, so further studies are necessary to evaluate its pathogenicity in intensive production animals, where microbiota imbalance can be decisive in the development of opportunistic diseases.

Acknowledgments

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Development of ELISA protocol for detection of sIgA against *Mycoplasma hyopmeumoniae* in pigs from a commercial kit

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Introduction

Mycoplasma hyopneumoniae is the main causative agent of enzootic pneumonia (EP), a chronic respiratory disease in swine, and one of the major pathogens involved in the respiratory disease complex (PRDC) (1). Considering that about 70% of the world's pig herd carries out vaccination against the pathogen (2), serological differentiation between vaccinated and infected animals is difficult. Secretory IgA (sIgA) is a crucial factor in the protection of pigs against M. hyopneumoniae and the main effector of respiratory tract mucosal immunity (3). Feng et al. (2010) (4) states that it is possible to differentiate vaccinated animals from infected animals by the detection of sIgA, since systemic vaccination generally promotes little stimulation of mucosal immunity (5). Since IgA is a response related to the first contact of the pathogen with the respiratory mucosa, the detection of sIgA can be used to support the diagnosis of the disease (6). Thus, this work aimed to the development of an ELISA protocol for detection of sIgA anti-*M. hyopneumoniae* in samples of nasal swabs of pigs by the adaptation a commercial kit..

Materials and Methods

A commercial ELISA test was used with some modifications. Initially, the plate was blocked with *PBS* 1.5% ovoalbumin, followed by incubation at 37°C for 30 min. Samples of nasal swabs from 20 24-day-old piglets free of *M. hyopneumoniae*, followed by experimental infection, were homogenized as a pool and used as negative (NC) and positive controls (PC). To validate the results, nasal swab samples from different pigs, previously tested positive by qPCR, were also evaluated. Swabs were deposited in graduated microtubes containing 500 µL of *Phosphate Buffered Saline (PBS)* 1x concentration, pH = 7.4, and stored in a freezer at -20°C until analysis. The samples to be tested were rapidly homogenized in vortex and 100 µL of the liquid fraction were deposited in each well, as well as the controls, tested in duplicate. The samples were incubated for 60 min at room temperature (RT). The conjugate was replaced with a goat anti-Pig IgA Antibody HRP Conjugated (Bethyl Laboratories Inc.), at the dilution of 1:500 using the diluent provided by the kit. The conjugate was incubated for 60 min at RT. The washing procedures and all subsequent steps were performed according to the commercial kit protocol. In all plates, the conjugate was tested separately to determine the adsorption thereof in the absence of sample. The OD$\bar{x}$ values obtained from the conjugate were deduced from the OD values of the samples. This test was performed twice to evaluate its repeatability. The plate reading was performed on an absorbance microplate reader (iMark, Bio-Rad Laboratories Inc.), filter 655.

Results

The mean optical densities (OD) found for negative and positive controls ($PC\bar{x}$; $NC\bar{x}$) were calculated for the determination of S/P values (sample/positive ratio) according to formula: S/P = $OD - NC\bar{x}/PC\bar{x} - NC\bar{x}$. The S/P threshold between the positive and negative samples was calculated from the S/P $NC\bar{x}$ ± 2x standard deviation. The results obtained from the repetitions were similar.

Conclusions and Discussion

The OD$\bar{x}$ values obtained from the conjugate were deduced from the OD values of the samples, as it was nonspecifically bounding to the wells. This may have occurred because of the higher concentration of the conjugate, as well as the antigen content used to sensitize wells. Differences between S/P values of negative and positive controls were statistically significant ($p<0.05$). The S/P values of samples from positive pigs in qPCR were also statistically different from the negative control ($p<0.05$). The similar results obtained in the repetitions shows the reliability of the results.

We concluded that the adaptation of a commercial kit for the detection of sIgA anti-*M. hyopneumoniae* in pig nasal swab samples was feasible and capable of providing reliable results.

Acknowledgments

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Estimation of the content of lean meat quality in pigs

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Introduction

Measurement of lean meat on slaughter line and formation of price on the basis significantly contribute to the overall improvement of the quality and profitability of production and distribution of pork. Determination of lean meat content on carcass is measured by different electronic-optical devices, such as PIGLOG 105, Fat-O-Meater (FoM), and other methods like „methods two points", partial dissection, total dissection and others (1,2). Therefore, the objective of the present study was to determine the content of lean meat on carcass in slaughterhouse using three diferent methods (FoM, two points and partial dissection) and compare to results of content of lean meat on live farm pigs (PIGLOG 105).

Materials and Methods

Pigs originated from a commercial farm which produced 40.000 finishers per year. In this study, pigs were chosen randomly, after that pigs were adequately tagged in order to follow traceability in the chain until the end of the measurement in the slaughterhouse. The content of lean meat on live pigs was measured on the farm using ultrasound device PIGLOG 105, while in slaughterhouse, the content of lean meat was measured using FoM, methods two points and partial dissection.

The results were analyzed statistically, taking into consideration arithmetic means, standard deviations, coefficients of variation, and coefficients of simple correlation. Furthermore, the basic ANOVA model was performed using the LSD procedure.

Results and Discussion

Forty years ago, some countries used the sonographic apparatus for carcass quality evaluation (3), while in Serbia there is still Rulebook on the Quality of Slaughtered Pigs and Pork Meat Categorization. Even though in Serbia the grading of pig carcasses was not obligatorily performed based on the SEUROP system, slaughterhouses which measure the content of lean meat, use this classification. The carcasses are graded according to the content of lean meat and carcass weight. Farmers often did not believe the results of percentage of lean meat from slaughterhouse, especially when they received payments for live pigs, based on results from slaughter line. These results show how farmers can control percentage of lean meat on farms and compare with results from slaughterhouse. The content of lean meat is presented in Table 1, for each methods measure.

Table 1. Summary Statistics of lean meat content

	Aver %	*Stand dev*	*Coeff. of variation %*	*Min %*	*Max %*
PIGLOG-105	59,3	2,532	4,270	53,3	63,5
FoM	54,4	2,848	5,232	49,2	61,6
Two points	55,3	4,480	8,105	42,1	60,8
Partial dissection	58,4	3,068	5,255	48,4	64,6
Total	56,9	3,871	6,809	42,1	64,6

Regardless of the method for measuring the content of lean meat before and after slaughter, results have to be the same, as it has already been described in previous research (4).

Conclusions

Farmers should get feedback from slaughterhouse about the quality of their pigs, improve genetics, diet, conditions of keeping pigs, and check percentage of lean meat on farm, in order to avoid possible litigation and court case.

Acknowledgments

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Influence of fever on udder temperature variations assessed by infrared skin thermography

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Introduction

Fever is a physiopathological event related to a wide range of diseases or other phenomena such as vaccination by means of immune stimulation[1]. There is a lack of literature describing the effect of the increase in body temperature and its relation to the temperature in the mammary gland. Therefore, the aim of this study was to investigate the thermal variations in the udder of sows during the suckling period in animals with or without fever using infrared skin thermography (IST), to know if there are differences between anterior and posterior teats and to evaluate the association of fever and udder temperature of the sows.

Materials and Methods

The trial was performed on a 3,300 sows farm in Goiás state (Brazil). Rectal temperature (RT) and IST of 31 sows were measured twice a day for 5 consecutive days in the middle of suckling period, in the position in which the animal was found in the farrowing crate (standing or lying). RT was assessed by an electronic clinical thermometer. Fever events were considered to be present when an RT value $\geq$ 40ºC was recorded. Thermographic images were obtained using a FLIR OnePro Thermalcamera for Android (Flir, USA), connected to a smartphone. The thermographic images were analysed by looking at the maximum (Tmax), mean (Tmean) and minimum temperature (Tmin) taking the udder as a whole, and the same parameters for the anterior (TA) and posterior (TP) teats, dividing the udder through the middle. The differences in the parameters were assessed by Student's t test and correlated by means of Pearson correlation.

Results

One hundred and ninety-seven RT and thermographic image data were analysed.

The 38 RT data with fever showed a significantly higher temperature than the 159 RT data without fever, with a mean significant difference of 1.2ºC. A similar difference in mean temperature was detected when analysing the IST in the udders of animals with and without fever (Table 1). However, the maximum temperature in the udders was higher than the maximum RT in the animals.

Table 1. Mean rectal temperature of sows and infrared skin thermography of udders classified by fever.

	Fever		Dif	p-value
	No	Yes		
RTmean	39.1±0.53	40.3±0.6	1.2	P<0.001
Tmax	41.42±1.13	42.43±0.9	1.01	P<0.001
Tmean	36.22±1.4	37.17±1.5	1.48	p<0.001
Tmin	25.90±.1.6	26.74±1.3	0.84	P=0.002
TAmax	41.23±1.09	42.27±1	1.04	P<0.001
TAmean	36.43±1.6	37.57±1.7	1.14	P<0.001
TAmin	27.06±2.3	27.72±1.4	0.66	NS
TPmax	41.06±1.1	42.13±0.9	1.13	P<0.001
TPmean	36.60±1.5	37.3±1.4	0.7	P=0.009
TPmin	26.57±2.2	27.2±1.5	0.63	NS

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With regard to the correlation between the different parameters, different correlations were obtained between RT and the parameter in the mammary glands. Maximum temperatures in the udders showed the highest correlations with RT (RT vs Tmax r=0.512, p<0.001; RT vs TAmax r=0.514, p<0.001 and RT vs TPmax r=0.534 p<0.001).

Conclusions and Discussion

Fever is correlated to the increase in temperature in the udder, the increase being similar in the anterior and posterior mammary glands. Udder temperatures recorded by other authors using IST were lower than RT[2], however, this study showed a higher temperature in the IST for Tmax, TAmax and TPmax than RT. The correlation found for Tmax, TPmax and TAmax with RT is similar to others previously recorded[3]. The increase of temperature in mammary glands could influence the performance of piglets during suckling. The mammary gland could be a thermal window to assess the fever by means of IST, since it is correlated the IST and the RT.

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Occurrence and impact of ear tip necrosis on daily weight gain in weaned piglets

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Introduction

Ear tip necrosis (ETN) is characterized by a blue to black discoloration of the ear tips followed by necrosis (1). It often occurs in piglets of one to ten weeks (4). Different risk factors have been described such as mycotoxins in the feed, ear biting, high stocking density, poor ventilation and drafts, low temperature in the stable, mange or insufficient enrichment of the environment (1,2,3,4). The present study investigated the prevalence of ETN and its effect on daily weight gain (DWG) in nursery piglets.

Materials and Methods

A Belgian herd with ETN in nursery piglets was selected. Four consecutive groups (A,B,C,D) of 580 to 680 weaned piglets were followed. The pigs were housed for seven weeks in mechanically ventilated rooms with partially slatted floor (0.3 m^2/pig). They were fed *ad libitum* with a commercial dry feed (meal).

The presence and severity of ETN was investigated weekly on all pigs. Severity was quantified with scores ranging from I to IV. Score I: small crust on ear tip; score II: small wound on ear tip with reddening around; score III: bloody, necrotic wound on ear edge; score IV: partial lack of auricle with necrotic edge. Mortality and antimicrobial usage were also monitored. Individual weighing of pigs was done on 8 randomly selected pens (group C and D; 199 pigs in total).

Results

The weekly prevalence of ETN in the four batches of nursery pigs (including scores I to IV) increased with weeks post-weaning, especially from week 5 post-weaning onwards (Table 1).

Table 1: Weekly prevalence of ETN (% affected pigs) in the four batches (A to D) of weaned pigs

Week post weaning	Weaning batch			
	A (n=583)	B (n=547)	C (n=676)	D (n=672)
1	0.3	0.2	0.1	0.0
2	0.3	0.9	0.1	0.9
3	0.9	2.0	0.7	0.9
4	1.9	10.1	4.7	4.6
5	12.5	20.3	7.2	10.2
6	23.8	23.6	10.7	16.8
7	21.8	31.6	11.1	23.7

From the animals with lesions, the percentage for which both ears were affected was 45%, 38%, 30% and 40%, in batch A, B, C and D, respectively. The percentage of lesions scored I, II, III and IV at the end of the nursery was 84.3%, 14.4%, 1.1% and 0.0%, respectively.

The DWG of affected and non-affected pigs was 394 and 406 g/day, respectively (Table 2). From the pigs with lesions, DWG of pigs with either one (n=47) or both ears affected (n=13) was 400 and 373 g/day, respectively ($p>0.05$).

Table 2: DWG (g/day) of nursery pigs (n) with or without ETN in a subsample of batches C and D

Weaning batch		C	D	C+D
ETN	Yes	378 (n=25)	417 (n=35)	394 (n=60)
	No	394 (n=64)	416 (n=75)	406 (n=139)
P-value		0.24	0.96	0.43

Mortality was 0.8%, 2.3%, 1.5% and 1.0% in weaning batch A, B, C and D, respectively. The percentage of days that pigs received antimicrobial medication during nursery period was 25, 37, 44 and 29 for batch A, B,C and D, respectively. Medication was mainly used against *E. coli* and *S. suis* infections.

Conclusions and Discussion

The prevalence of ETN increased with time post-weaning, and especially during the last weeks of the nursery. The percentage of affected pigs at the end was high and varied between batches (11% to 32%). Most affected piglets (84%) had mild lesions (score I) and 38% of the affected pigs had lesions on both ears.

The DWG of affected pigs was not significantly lower compared to non-affected pigs, although DWG of pigs affected on both ears was 33 g/day less than non-affected pigs. This might be due to the large variation in DWG and the fact that lesions mainly occurred towards the end of the nursery. Further research including more batches and assessing control measures is ongoing.

Acknowledgements

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Evaluation of systemic and local tolerance of intramuscular administration of a toltrazuril and iron suspension (Baycox® Iron Injection), compared to standalone iron administration

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Introduction

Suckling piglets undergo different management procedures during the first days of life. Parenteral (intramuscular) iron administration is a common practice for the prevention of iron deficiency anaemia (IDA) in newborn piglets (1, 2). In 2019, a patented, injectable combination of toltrazuril and iron (Baycox® Iron Injection, Bayer Animal Health) has been introduced in Europe (3). The combined application of iron and toltrazuril reduces handling of piglets and the number of interventions they are submitted to, optimizing the management practices and improving animal well-being. The objective of this study was to evaluate the systemic and local tolerance of intramuscular administration of a toltrazuril and iron suspension (Baycox® Iron Injection), compared to standalone iron. This was performed by clinical observations post-treatment and histological evaluation of the application site.

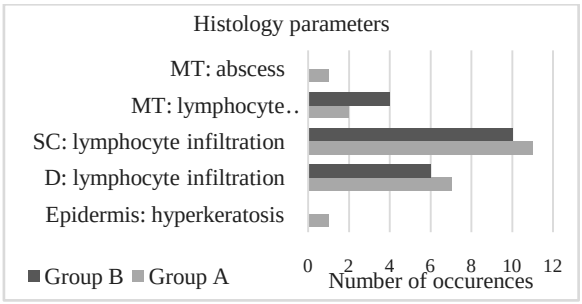
Materials and Methods

On the third day of life (SD3), 24 piglets were ranked by body weight (BW) and randomly allocated to two treatment groups. Control group A (n = 12) received 200 mg/animal of iron, as gleptoferron (Ursoferran®), while group B (n = 12) received a combination of 20 mg toltrazuril/kg BW and 100 mg iron/kg BW, as gleptoferron (Baycox® Iron Injection). Treatments were administered intramuscularly, in the neck area (left side) behind the ear using a disposable 21G needle and the site was permanently tattoo marked for further evaluation. Assessments for injection site reactions and other adverse effects were conducted within one hour before as well as 3, 6, 24, 48 and 72 hours after treatment. All piglets were observed daily for general health throughout the study.
At the end of the study (SD30) all piglets were humanely euthanized, and the injection site was examined by histopathology (fixation, paraffin embedding, H&E staining of sections) at the Institute of Pathology, Vetmeduni Vienna. Lesions at the injection sites (abscesses, lymphocyte infiltrations in the muscle tissue/subcutis/dermis, hyperkeratosis) were evaluated microscopically in a semi-quantitative manner.

Results

Minor health observations (e.g., bite wounds), but no major adverse events were seen. All of the observations were classified as unrelated to treatment administration. Except for one animal in control group, no macroscopic lesions were observed at the injection site at the time euthanasia was conducted.
In the histological analysis of injection sites, the epidermis was unaltered except for one animal in the control group, which showed low-grade hyperkeratosis. In the dermis, subcutis and the underlying muscle tissue, lymphocyte infiltrations were noted in both groups at different grades. In the control group, one animal had an encapsulated abscess. Mild dermatitis was registered in both groups, but the associated lymph nodes were inconspicuous in all groups. Overall, 22 lesions were recorded for the control group A (n=12) and 20 for group B (n=12) over five parameters (Fig. 1).

Fig. 1: Count of histological lesions at the end of the trial (30 days of life, A: 12 piglets, B: 12 piglets)



MT = Muscle tissue; SC = Subcutis; D = Dermis

Conclusions and Discussion

No external injection site and adverse reactions were noted in relation to treatment. Histology of the injection site revealed no clinically relevant lesions except for an encapsulate abscess in one piglet of the control group A. Overall, signs of mild non-purulent dermatitis as a reaction to the injection and the associated mechanical trauma were noted in both groups but without clinical manifestation.
In conclusion, the injectable combination of toltrazuril and iron (Baycox® Iron Injection) proved to be systemically and locally safe, causing no injection site macroscopic lesions and similar histopathological lesion profile as routinely used standalone iron.

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Detection of pseudorabies virus in oral fluid specimen using real-time PCR

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Introduction

Pseudorabies virus (PRV) causes disease of the central nervous system and respiratory/reproductive signs. Many countries have eliminated PRV from domestic swine, but the virus remains endemic in feral swine and is occasionally introduced into commercial herds. In China, highly pathogenic PRV variants have been described and there are concerns regarding its spread to other countries (1, 2). For these reasons, PRV detection systems for control and elimination are still needed. The objective of this project was to evaluate the detection of PRV in swine oral fluid collected from vaccinated and/or inoculated pigs using real-time PCR assays targeting PRV gB and gE genes.

Materials and Methods

Samples of known PRV infection status were used to evaluate the assays and establish shedding dynamics. Nasal swabs and oral fluid samples were collected from 40 12 - 16-week-old pigs in 4 treatment groups (n = 10 per group; Table 1). Pigs in Group 1 and 2 were vaccinated with a commercial vaccine (Ingelvac® Aujeszky MLV). Pigs in Group 1 and 3 were intranasally exposed to a classical strain of PRV (PRV 3CR Ossabaw). To detect the presence of PRV DNA, samples were tested using 1) gB PCR for screening PRV-positive animals and 2) gE triplex PCR for differentiation between PRV classic and PRV highly pathogenic (variant) strains.

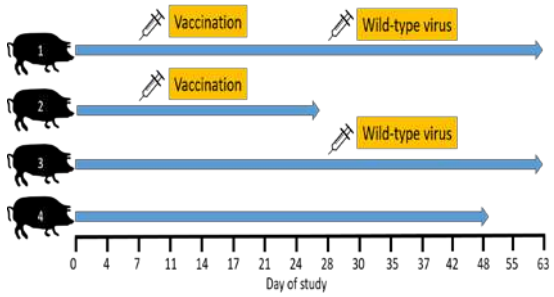

Figure 1. Experimental design for creating nasal swab and oral fluid specimens of known PRV status.

Results

The gB PCR detected PRV DNA in oral fluid samples within 24 hours post vaccination (8 DOS) and/or inoculation (29 DOS) (Table 1). False positive results (n= 4) were observed in nasal swab samples on the day of vaccination (7 DOS, Groups 1 and 2). Vaccine and wild-type viruses were detected up to 2 and 30 days post vaccination or inoculation, respectively. The post-inoculation detection rate was lower and the shedding period shorter in vaccinated pigs (Group 1). Shedding patterns in oral fluid were comparable to nasal swabs. Compared to the gB PCR, PRV classic virus samples had fewer positives and for a shorter period of detection 37 to 47 DOS in oral fluid and 30 to 42 DOS in nasal swabs (Group 3). No classic PRV was detected in vaccinated and negative control pigs (Group 1, 2, and 4). No (false) positive results for the highly pathogenic PRV variant were observed with the triplex gE PCR in nasal swab or oral fluid samples.

Conclusions and Discussion

PRV DNA was detected in oral fluid samples from PRV vaccinated and/or inoculated animals using real-time screening PCRs targeting gB and gE genes. The gB PCR results were consistent with a previous report on PRV detection in swine oral fluid samples from pigs inoculated with wild-type virus (Panyasing et al., 2018). Given that false positive and false negative results were observed in both nasal swab and oral fluid specimens, future research should focus on the improvement of the diagnostic specificity and analytical sensitivity of both PCRs.

Table 1. Oral fluid PRV detection time based on gB and gE PCR testing.

Group	PCR	1st detection (DOS*)	Last detection (DOS)
1	gB	8	36
	gE (classic)	NA	NA
	gE (variant)	NA	NA
2	gB	9	9
	gE (classic)	NA	NA
	gE (variant)	NA	NA
3	gB	30	58
	gE (classic)	37	47
	gE (variant)	NA	NA
4	gB	NA	NA
	gE (classic)	NA	NA
	gE (variant)	NA	NA

* DOS = day of study; * NA = negative to target genes

Acknowledgments

Funding provided by the Swine Health Information Center, Ames, IA

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Microgranulated premixes ensure superior clinical efficacy

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Introduction

The formulation of a medicated premix determines to a large extent the clinical outcome of an antimicrobial treatment. The clinical efficacy of 2 premixes, both containing the same active compound at the same concentration, was evaluated.

Materials and Methods

Pigs (n=32) free from *Actinobacillus pleuropneumoniae* were divided in 4 groups. Three groups were intranasally challenged with *Actinobacillus pleuropneumoniae* serovar 7 (2 x 10^8 CFU, tiamulin MIC 16 µg/ ml). Two groups were infected and treated with 2 different premix formulations, both containing 100 g tiamulin hydrogen fumarate/ kg: a powder based formulation and Vetmulin® microgranulated premix (Huvepharma®). The unique microgranulation technology of Vetmulin® premix ensures superior homogeneity of the active ingredient in the medicated feed and logically, results in correct dosing of all treated pigs. The pigs in both groups were treated at 10 mg tiamulin hydrogen fumarate/ kg bodyweight/ day for 15 consecutive days, starting 18 hours after the challenge. An uninfected and an infected control group without treatment were also included. The clinical efficacy was evaluated daily by 3 parameters: a respiratory score, a decrease of locomotory activity score (0 = no signs observed→ 4 = severe symptoms) and the number of days with fever (>39.8°C). The evalu ation was based on the average results over the 15 days treatment period.

Results

When comparing the Vetmulin® group with the powder formulation group, a significant reduction ($p<0.05$) of 83% of the respiratory score (0.10 and 0.57 respectively, fig. 1) and 93% of the decrease of locomotory activity (0.03 and 0.43 respectively, fig. 2) was observed. Furthermore, the number of days with fever was 0.6 days less in the Vetmulin® group (5.80 and 6.40 days respectively, fig. 3).

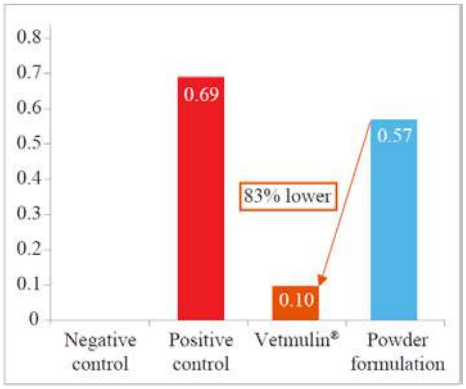

Figure 1. Respiratory score.

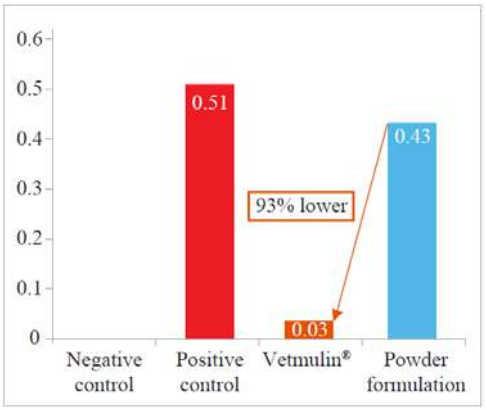

Figure 2. Decrease locomotory activity score

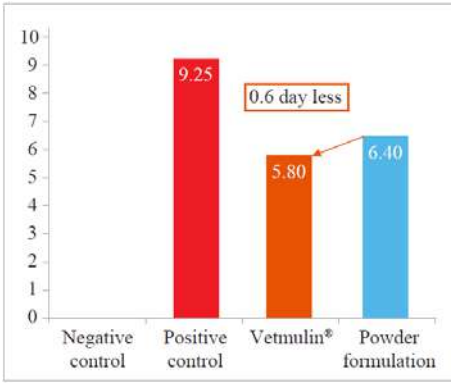

Figure 3. Number of days with fever

Conclusions and Discussion

The formulation of a medicated premix determines to a large extent the clinical outcome under field conditions. The superior homogeneity of Vetmulin® microgranulated premix in medicated feed results in an optimal efficacy.

Optimal tilmicosin absorption ensures superior clinical efficacy

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Introduction

A previous comparative study showed that the tilmicosin absorption rate and speed of Tilmovet® 200 g/ kg premix (Huvepharma®) was higher and faster than another 20% tilmicosin containing premix (1). After a treatment with 16 mg tilmicosin/ kg bodyweight, plasma concentrations were measured at 12 time points for 48 hours. The area under the curve (AUC), the maximum reached plasma concentration (Cmax) and the time to reach this maximum plasma concentration (Tmax) were determined and compared. In the Tilmovet® group the values of AUC and Cmax were respectively 17.3% and 20.2% higher and Tmax was achieved 17% earlier. The impact of this difference in pharmacokinetic behavior on the clinical efficacy was investigated in a clinical challenge model.

Materials and Methods

Pigs (n=32) free from *Actinobacillus pleuropneumoniae* were divided in 4 groups. Three of them were intranasally challenged with *Actinobacillus pleuropneumoniae* serovar 7 (2 x 10^8 CFU, tilmicosin MIC 16 µg/ ml). Two groups were infected and treated with Tilmovet® or the other 20% tilmicosin containing premix. For both groups, tilmicosin was administered at a daily dose of 16 mg/ kg bodyweight for 15 consecutive days, starting 18 hours after the challenge. An uninfected and an infected control group without treatment were also included. The clinical efficacy was evaluated daily by 3 parameters: a respiratory score, a decrease of locomotory activity score (0 = no signs observed→ 4 = severe symptoms) and the number of days with fever (>39.8°C). The evaluation was based on the average results over the 15 days treatment period.

Results

When comparing the Tilmovet® group with the other group, a significant reduction ($p<0.05$) of 62% of the respiratory score (0.28 and 0.74 respectively, fig.1) and 43% of the decrease of locomotory activity (0.17 and 0.30 respectively, fig.2) was observed. Furthermore, the average number of days with fever was 1.1 days less in the Tilmovet® group (6.40 and 7.50 days respectively, fig.3).

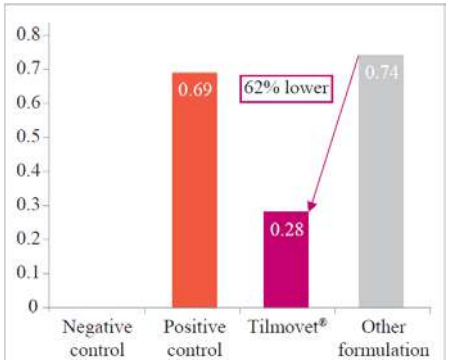

Figure 1. Respiratory score

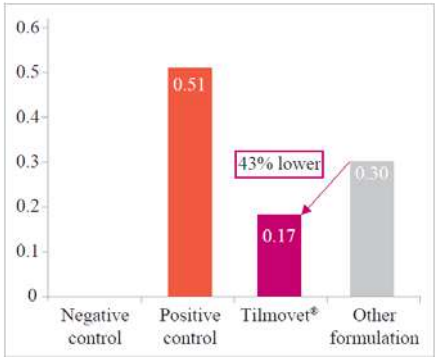

Figure2. Decrease locomotory activity score

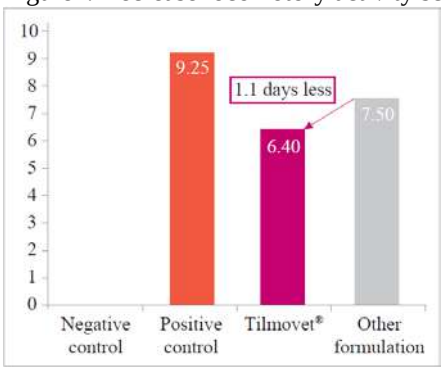

Figure3. Number of days with fever

Conclusions and Discussion

The absorption rate and speed of the active ingredient in the intestinal tract determines the clinical outcome of a treatment to a large extent. The superior absorption and distribution in lung tissue of Tilmovet® premix results in optimal clinical efficacy.

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Oral fluid sampling at the abattoir − an alternative to on-farm sampling?

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Introduction

Monitoring pig populations with the objective of declaring a farm, region, or country free of a particular disease requires extensive sampling(1). Currently, conventional monitoring practices require multiple visits to collect either individual samples (e.g., swabs, blood)(2), or aggregate samples (e.g., oral fluids)(3). This on-farm approach requires time, money, and poses a risk to biosecurity. Alternatively, the abattoir is a point of concentration of market pigs that could be used to easily surveil a specific farms, systems, and regions. The objective of this study was to determine whether surveillance could be done using oral fluid samples collected at the abattoir. Porcine reproductive and respiratory syndrome virus (PRRSV) and Senecavirus A (SVA) were used to represent endemic and emerging pathogens, respectively.

Materials and Methods

Oral fluid (n = 10) and serum (n = 10) samples were collected from 36 groups of finishing pigs within 48 hours of transport from the farm to the abattoir. At the abattoir, oral fluids (n = 3) were collected from the same groups while held in lairage. Samples were tested for the presence of PRRSV RNA or SVA RNA by rRT-PCRs and for PRRSV or anti-SVA antibody by ELISAs. If one sample from the group tested positive by a test, the group was considered positive for that respective pathogen. The frequency of detection was analyzed by individual samples and by group. Group results were used to report the general agreement between farm and abattoir.

Results

Oral fluids were successfully collected from 32 of the 36 (89%) groups at the abattoir. A total of 316 serum, 319 on-farm oral fluids, and 96 abattoir oral fluids were available for testing.

On the basis of individual results, PRRSV antibodies were detected in 295 (93.4%), 319 (100%) and 96 (100%) of serum, on-farm, and abattoir oral fluids, respectively. PRRSV RNA was detected in 6 (1.9%) of the sera, 55 (17.2%) of on-farm oral fluids, and 17 (17.7%) of abattoir oral fluids. No SVA antibodies were detected in sera or on-farm oral fluids, and no SVA RNA was detected in on-farm oral fluids, but 55 (57.3%) of abattoir oral fluids were positive on SVA rRT-PCR.

On the basis of group results, all groups were PRRSV ELISA positive on serum, on-farm, and abattoir-oral fluids. By PRRSV rRT-PCR, 16 (50%) of the groups were positive on oral fluids collected on the farm and 9 (28.1%) were positive based on abattoir oral fluid samples. All groups were SVA-negative based on on-farm oral fluid rRT-PCR testing. However, 21 (65.6%) groups had at least one sample test SVA RNA positive. Sixteen of 32 (50%) groups had the same results at the farm and at the abattoir (7 positive (21.9%), 1 suspect (3.1%) and 8 negative (25%) groups); the other 16 groups had different group results for PRRSV rRT-PCR results on oral fluid samples collected on-farm and abattoir oral fluids. All groups were considered to have different results for SVA RNA when comparing on-farm and abattoir oral fluid results.

Conclusions and Discussion

Oral fluids were successfully obtained at the abattoir while pigs were in lairage. A high number of PRRSV ELISA-positive samples was expected because the majority of the hogs were vaccinated with a modified-live virus vaccine and were raised in swine-dense regions.

The lack of positive SVA PCR results on on-farm samples and the high number of positive results for abattoir samples suggests the possibility of environmental contamination of animals during transport or on the lairage area.

Oral fluids had a higher rate of PRRSV RNA detection than serum - which is in agreement with the literature.

The disagreement between on-farm and abattoir results can be explained in part by the fact that many more samples were collected at the farm (n=10) than at the abattoir (n=3). However, disagreement in SVA on-farm vs abattoir results needs further research.

In summary, surveillance base on swine oral fluids collected at the abattoir is an alternative to current practices, especially for antibody-based tests, and for non-endemic diseases. However, further studies are needed to understand the results of this study and to develop sample size guidelines.

Acknowledgments

The implementation of this project was possible due to support from Drs. Tara Donovan and Kate Dion from The Hanor Company, Dr. Deborah Murray from New Fashion Pork, and Dr. Barry Wiseman from Triumph Foods LLC.

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Swine qPCR testing: The importance of quantification and detection limit

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Introduction

The quantitative polymerase chain reaction (qPCR) detects and quantifies target nucleic acids using specific primers and probes and is widely employed by swine veterinarians. qPCR results are expressed in Cq values, which describe the number of amplification cycles before the target nucleic acid is detected. The lower the Cq, the higher the amount of pathogenic DNA or RNA in a tested sample.

In veterinary practice qPCR is used both for diagnosing disease, status and for evaluating vaccine efficacy. Since qPCR assays consist of two parts (nucleic acid extraction and PCR amplification) Cq values can vary significantly between tests. The poorer the extraction the higher the Cq. In order to accurately estimate the load of a given sample, the Cq result must therefore be compared with that of known standards, which are tested during the same run.

The detection limit of a qPCR test (AKA the lower limit of detection or LLOD) describes the minimum number of copies of target that can reliably be detected in a given reaction. This parameter is of particular importance when considering the number of samples that can be pooled before a pathogen will no longer be detected. BioChek always determines the LLOD when designing a PCR kit and releasing production batches. Using relevant examples, this abstract describes: 1) How quantification is performed; 2) How LLOD is determined; and 3) How LLOD determination is critical for ensuring that infections are accurately detected and vaccine efficacy is reliably evaluated.

Materials and Methods

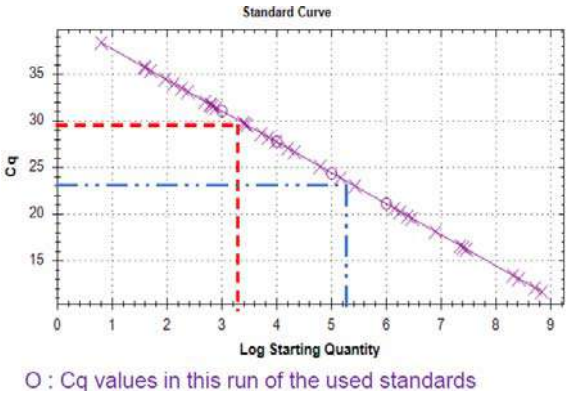
1.Quantification of target copy number: Cq values are measured using 4 defined standards (10^3, 10^4, 10^5 and 10^6 copies/reaction). Observed Cq values are plotted against copy number on a log axis to produce a standard curve. The standard curve is then used to infer copy number from the observed Cq value of a sample of unknown concentration (Graph 1).

2. LLOD determination: During R&D BioChek tests the LLOD of final design batches multiple times using a serial dilution of target, ranging from 6, 12, 25, 50, 100, 250, 500 and1000 copies per reaction .

3. Example calculation of the acceptable number of samples that may be pooled: Based on the LLOD for the PRRS qPCR test, the acceptable number of samples that may be pooled is calculated [1,2,3] (Table 1).

Results

1. Bacterial load calculation using a standard curve.



Graph 1: BioChek APP PCR results SRP2 kit

In this run: Sample with Cq 30 has a 3.35 $\log_{10}$ load
Sample with Cq 23.5 has a 5.3 $\log_{10}$ load

2. LLOD determination of a PRRS qPCR test batch.

LLOD test results of multiple runs of a Prototype PRRS qPCR (R) batch varied for EU genotype from
6 – 50 and for the US genotype from 12 - 50 copies/reaction

3. Pooling amount still able to detect PRRS virus.

-Minimum serum load of infected animals after vaccination is often around ± 4 $\log_{10}$/ml [1,2].

-PRRS positive semen can already infect gilts at concentrations of 40 TCID50/ml to 400TCID50/ml [3]

Table 1: Calculation of acceptable numbers of samples for pooling of serum samples.

Example pool size calculation	serum (10.000 copies/ml)	nr. of copies present	samples/pool (LLOD 50)
volume used for DNA/RNA extraction	200 µl	2000	
Eluate volume (extracted DNA/RNA)	50µl	2000	
volume eluaat used for the reaction	5 µl	200	**4** (= 200/50)

Note it is not advised to pool extractions from semen samples since a viral load of pooled semen can end up below the LLOD of the qPCR test resulting in a false negative outcome.

Conclusions and Discussion

Quantification of the pathogenic load of a sample is often necessary in determining the clinical impact of infections, such as PRRS and PCV2, and in evaluating vaccine efficacy. Here we highlight the importance of using standards of known concentration to calculate the load of a given sample. We also highlight how pathogenic load and comparison with the LLOD of a given test is crucial in determining the suitability of pooling when testing.

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Feeding Behaviour in Ontario Nursery and Finishing barns: Preliminary Results from a Pilot Study

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Introduction

Health challenges on many farms occur at the time of weaning as evidenced by the increase use of antibiotics and zinc in early nursery rations (1). It is critical that recently weaned pigs have proper access to feed after weaning to avoid post-weaning health challenges (2). Other challenges such as environmental, social, health, diet, and feeder size may affect pig health and productivity on farm.

Recommendations for the appropriate ratio of pigs per feeder space vary considerably in Ontario (3). Many studies are outdated, no longer reflecting today's feeders, diets and genetics lines that are used in commercial facilities. With the inconsistent recommendations, observing behaviour around different pig-to-feeder space ratios may improve feed intake and animal health on farms thus decreasing antimicrobial usage. The objective of this project was to video record and analyze feeding behaviour around various feeder space ratios in Ontario nursery and finishing barns.

Materials and Methods

Video cameras were used in 11 finishing barns and 14 nursery barns between April and September 2019. Images were recorded at 30 second intervals (Figure 1), producing a time lapse video over 24 hours. Cameras were set up in barns for a period ranging from 24 hours to 6 days. After cameras were retrieved, analysis of footage was completed by quantifying number of pigs at the feeder every 60 seconds. This was done over a 24 hour period. 10 minute time intervals were then averaged for a 24 hour time period. Evaluations on patterns were done by graphing the 10 minute averages. Currently, 13 farms have been analyzed as analysis is still taking place.

Figure 1: Image snapped of pigs eating in a nursery pen.

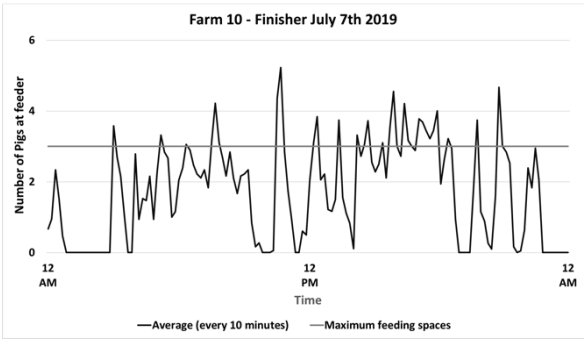

Figure 2: Graph of analysis on a finishing barn with a 10:1 ratio.

Results

There was a consistent pattern seen of weaned pigs eating in large groups. Pigs per feeder space ratio ranged from 3 to 7 pigs/feeder space. Approximately every hourly, nursery pigs went up to the feeder as a group, ate and left together. Two finishing pens in a 6:1 ratio only ate approximately 9 hours throughout the day during a 24 hour period while pigs in bigger ratios, such as 10:1 and 9:1, ate throughout the day and night during a 24 hour period (Figure 2). Finishing pigs were seen to adjust their daily behaviour, such as eating and sleeping, to their pig per feeding space ratio. Case management was also done on several farms.

Discussion

Nursery pigs observed eating in groups suggest that providing a larger number of feeder spaces may satisfy their social habits and eating behaviours. This implies that identical ratios may not provide equal access to feed because pigs may eat in groups, which could decrease productivity and health in barns. Larger finishing pig to feeder space ratios may result in pigs having less opportunity to access the feeder than smaller ratios. This data could suggest that pigs are adjusting their behaviour to their opportunity to access feed. The cameras are also a useful tool for disease diagnostics and production management. Observing and understanding pig behaviour around the feeder could optimize feed access. Improved feed access may increase productivity, animal health and welfare on farms, while decreasing the use of prophylactic or therapeutic antibiotics.

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Productive impact of sow vaccination during lactation on sow and offspring performance

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Introduction

Current swine production systems are achieving remarkably high numbers in piglets/wean/sow per year. This high performance of the sow during the lactation period is potentially due to genetic, feed, health, and management improvements. However, despite these important efforts, post-farrowing vaccination in sows is routinely applied to protect the breeding stock against Parvovirus sp. and erysipelas[1].Therefore, there is a need to study the impact that this strategy may have on sow and piglet's production performance parameters when applied during their lactation period[2,3]. As a result, the objectives of this study were to evaluate the potential impact that vaccination during the lactation period may have in sows and their offspring's performance.

Materials and Methods

A total of 61 sows and 654 piglets from a farrow-to-wean farm in the North-East of Spain were included in the study. Sows and piglets were aleatory assigned into 2 vaccination protocol groups; 1) Vac: Sows vaccinated at day 10 (d10) post-farrowing with a bivalent Parvovirus sp. (PPV) and erysipelas (ERY) commercial vaccine. 2) No Vac: Sows were inoculated at d10 with 2 ml of a physiological saline solution (Table 1).

Table 1. Study groups, commercial vaccines used, and vaccine protocols.

Group	Vaccine	Vaccination protocol
Vac (n=386)	Bivalent PPV and ERY commercial vaccine	Vaccinated at d10
No Vac (n=268)	Control	No Vac

Temperature (Tª, ºC) and daily feed intake (DFI, Kg/day) were recorded daily in sows, weight was registered in piglets at birth (Bdw, Kg), day 10th (Bd10w, Kg), and weaning (Bdwe, Kg). Average daily weight gain was calculated at d10 of lactation or vaccination day (ADG-B:10, kg/d), and then at weaning (ADG-10:W, kg/d).

All results were analysed using R software. A linear mixed model with both fixed and random effects was applied to analyse the **average daily weight gain of piglets at weaning** through covariates such as the treatment group, daily weight gain at day 10, differences of temperatures at 6 and 24 hours after the vaccination, and the differences of intakes' percentages one, two and three days after the vaccination, as well as all possible interactions between all covariates.

Results

Results from variable modeled in the study are summarized in Figure 1. Results from the model showed that the average gain difference in the piglets **control group was 8 grs higher** than in the treatment group, and this difference was statistically significant (p-value=0.0411). However, no differences in temperatures and intake percentages after vaccination were found between such groups. Interactions between these covariates and the group factor were also not statistically significant.

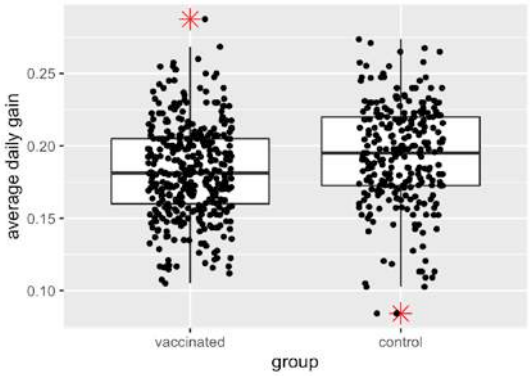

Figure 1. Boxplot with individual average daily gain (kg) data points from all piglets included in the study

Conclusions and Discussion

Results from this study demonstrated that vaccination protocols post-farrowing had an impact in average daily gain of piglets. However, under the conditions of this study, variables such us sow feed intake and temperature in sows were not significant potentially due to changes of the normal management practice during this period. Therefore, more studies are needed to understand the variables that may impact this performance difference.

Acknowledgments

The authors would like to thank the farmer and farm staff for their help during the study.

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Effects of dietary calcium on the structural and mechanical properties of the femur in adult obese Ossabaw miniature swine

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Introduction

The pig has become an increasingly popular model for human biomedical research due to their similarities to humans in body mass and genomic and metabolic profiles (1). Larger animal models can be more useful for biomechanical evaluation of various bone therapies, due to their skeletons bearing more similar mechanical loads to humans, compared to rodent models (2). As humans live longer due to general advances in medical care, the prevalence of hip fractures is increasing (3). Calcium deficiency in adults may be an important determinant for hip fracture incidence in the elderly (4). Calcium supplementation has been encouraged since the calcium obtained from daily food consumption is often below current dietary recommendations (5). Therefore, increasing calcium intake during adulthood and aging may help decrease the prevalence of hip fractures with aging. This study aims to determine how dietary calcium supplements from different sources may influence bone structural and mechanical properties in the femur of adult Ossabaw pigs.

Materials and Methods

Female Obese Ossabaw miniature swine (14-16-months-old; n=8/group, mean final body weight= 93.36kg) were fed one of the following diets for six months; Control $CaCO_3$ diet: 0.33% Ca (in total diet), High Supplement $CaCO_3$-based diet (HS): 1.9% Ca, High Supplement Dairy-sourced diet (Dairy): 1.9% Ca. Following the diet treatments and euthanasia, peripheral quantitative computed tomography was performed on the femoral neck, midshaft and distal metaphysis of the right femora. The right femoral neck was also examined by micro-computed tomography. Intact left femora were subjected to femoral neck fracture tests. Kruskal-Wallis and Mann-Whitney U tests were performed to test for differences between the diet groups ($p<0.05$).

Results

In the femoral neck (f), relative to the Control group, the Dairy and HS groups had greater total bone density (fTot.Den), total bone strength index (fTot.BSI), trabecular density (fTr.Den), bone volume fraction (fTr.BV/TV), apparent BMD (fTr.BMD), but lower trabecular area (fTr.Ar) (P= 0.043; 0.042; 0.022, 0.018, 0.023 and 0.048). In the distal metaphysis (d), total BSI (dTot.BSI) and trabecular BSI (dTr.BSI) were greater in the Dairy and HS groups than the Control (P= 0.024 and 0.029) (Table 1). The Dairy and HS groups did not differ in these measured properties. The structural and material differences between the supplemented Ca groups and the Control group did not result in distinguishable differences in the mechanical properties of the femoral neck. No measurable differences were present at the midshaft.

Table 1. Sub-set of femoral structural and material properties for the diet groups: Control, High Dairy-based Ca diet (Dairy) and High $CaCO_3$ diet (HS).

	fTot.Den (mg/cm^3)	fTot.BSI (mg*mm)	fTr.Den (mg/cm^3)	fTr.Ar (mm^2)
Control	588.78^a	113.03^a	484.62^a	206.76^a
Dairy	638.60^{ab}	137.57^b	524.65^b	191.16^a
HS	664.32^b	140.40^b	554.58^b	161.74^b
	fBV/TV	fBMD (mg HA/cm^3)	dTot. BSI (mg*mm)	dTr.BSI (mg*mm)
Control	0.332^a	379.38^a	293.39^a	259.98^a
Dairy	0.400^b	416.14^b	356.41^b	320.17^b
HS	0.412^b	430.00^b	369.01^b	330.56^b

Different superscripts (a,b) illustrate significant difference between the groups for each measure.

Conclusions and Discussion

Cortical-enriched bone sites were generally unaffected by altered calcium diet. However, both Ca-supplemented diets had beneficial effects on the structural and material properties of cancellous bone sites (femoral neck and distal metaphysis). No consistent difference existed between the two Ca-enriched groups, indicating the positive effects of a calcium-enriched diet in any form. Despite the differences in the structural and mineral properties of the femoral neck between the Ca-enriched and control groups, femoral neck mechanical properties did not differ by group; which may be related to the low sample sizes for these tests (n=6-7). In a translational sense, a general enrichment in the properties of cancellous sites is important given the propensity for these sites to fracture in human osteoporotic patients (6).

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Impact of vaccination on transmission of *Lawsonia intracellularis* in growing pigs

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Introduction

Lawsonia intracellularis (LI) is an intracellular bacterium and causative agent of proliferative enteropathy (PE; ileitis). Antimicrobial has been widely used to control PE. Changes on regulation for the use of antimicrobials as growth promoters will directly impact well-established strategies, what has driven implementation of vaccination strategies. Our objective was to study the spreading of *L. intracellularis* infection in naïve and vaccinated pigs.

Materials and Methods

A seeder-pig sentinel model was used (Figure 1). Ninety pigs were divided into 3 groups: orally vaccinated; intramuscularly vaccinated and non-vaccinated. Day 21 post-vaccination, 9 seeder pigs were challenged. Day 7 post-inoculation, seeder pigs were commingled as described in the Figure 1. Transmission rate and expected probabilities of shedding were assessed with the susceptible–infectious (SI) model. Animals were considered infectious when fecal qPCR was equal or more than 103 L. intracellularis/g of feces.

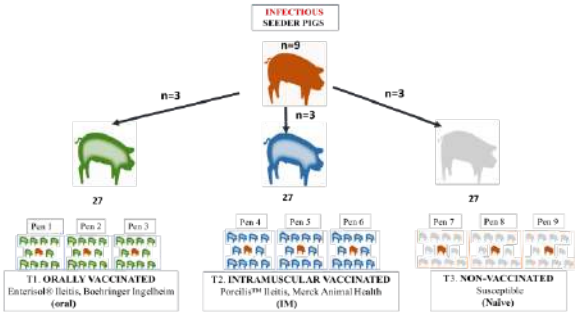

Figure 1 – Experimental design demonstrating the seeder-pig sentinel model.

Results

The transmission rate under a non-vaccinated scenario showed that one infectious pig (> 103) can transmit L. intracellularis to 3 susceptible pigs in per week.

Transmission rates were significantly reduced in both vaccinated groups: 1.8 infected pig per week in the orally-vaccinated and 1.7 infected pig per week in the intramuscularly-vaccinated group.

The period of fecal shedding was also decreased in both vaccinated groups. While the median period of shedding in non-vaccinated animals was 11.2 weeks, orally- and intramuscularly-vaccinated animals showed 6.3 and 8.3 weeks, respectively.

Conclusions and Discussion

These results highlight the importance of implementing vaccination programs at system level rather than site-specific interventions. This strategy prevents the risk of commingling batches of pigs from vaccinated and non-vaccinated sources, therefore reducing the impact of the disease in downstream flows.

Acknowledgments

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An association between lung lesions and growth performance in pigs

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Introduction

Economic losses associated with infections with *Mycoplasma hyopneumoniae* and/or *Actinobacillus pleuropneumoniae* can be attributed to reduced weight gain, decreased feed efficiency and extended fattening period. Postmortem examination of lung in slaughterhouse offers good opportunities for pig health and welfare monitoring through macroscopic identification of pathological lesions.

Lesions caused by *M. hyopneumoniae* infection are referred to as EP-like, as they are characteristic but not pathognomonic. *A. pleuropneumoniae* is one of the main pathogens associated with the development of pleuritis/pleuropneumonia (PP-like lesions).

The aim of the study was to determine the associations between EP-like and/or PP-like and their severity on defined markers of production performance.

Materials and Methods

A total number of 1976 pig lungs were scored. The study was carried out on 22 farrow-to-finish pig farms. From each farm batch of at least 75 fatteners was assessed in a slaughterhouse for the presence or absence of EP-like and PP-like lung lesions. EP-like lesions were scored according to Madec's grid and the Slaughterhouse Pleuritis Evaluation System for PP-like evaluation was used.

Individual carcass weights (post-trimming, hot weight) and lean meat percentage were collected for each pig. At the herd level slow growing pigs were defined as pigs which carcass weight was less by more than two standard deviations than the average in a given herd. Moreover *Actinobacillus pleuropneumoniae* Index (APPI) for each herd was calculated.

Pearson's correlation was calculated between lungs scores of EP-like/PP-like lesions and production performance parameters (carcass weight, lean meat percentage, slow growing pigs percentage) at the individual and herd level. The significance level was set at $p<0.05$ for all statistical analyses.

Results and Discussion

The severity of EP-like lesions (higher scores) in individual pigs, were significantly correlated with carcass weight ($p=0.002$). The correlation was negative and weak ($r=-0.084$). This result is in line with previously published results of similar studies. (1-4). Interestingly total score for pleuritis was weakly but positively ($r=0.079$) correlated with lean meat percentage ($p<0.001$). The explanation for this result is unclear but it might be related to the moment of *A. pleuropneumoniae* infection. High scores for PP-like lesions indicate acute infection in the final phase of fattening when fat deposition is the most intensive (5).

At the herd level strong positive correlation ($p=0.013$; $r=0.575$) was found for the percentage of slow growing pigs within batch and APPI. It might be assumed that slower grow of pigs is a consequence of chronic pleuropneumonia. An observation that chronic pleuritis in pigs is associated with scores ≥ 2 was made previously (6).

Conclusions

The results of this study highlight that EP-like lesions and pleuritis are routinely identified at slaughter and are significantly associated with a reduction in carcass weight and increase of slow growing pigs percentage within batch. The obtained results confirm that the presence of these lesions at slaughter is associated with a significant decrease in production performance which can result in substantial economic implications for producers.

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Farm questionnaire and evaluation of lung lesions at slaughter as a diagnostic tool in pigs health monitoring

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Introduction

Quick, inexpensive and reliable tools for evaluation of pigs health status may improve the diagnostics and are extremely helpful in decision-making process for effective disease control. The aim of the study was to evaluate the usefulness of post mortem evaluation of lung lesions to estimate health status of the farm and to find out the relationship between lung lesions and the farm questionnaire outcomes. Moreover we want to popularize non-invasive assessment methods that enrich the diagnostic process.

Materials and Methods

The experiment was carried out on 22 farrow-to-finish pig farms, selling at least 75 fatteners in each technological group. Antibiotics were not administered through the last 2 weeks before slaughtering. In the first stage of the study the farm questionnaires were performed. The survey consisted of 67 more or less detailed questions and it was completed during the veterinarian visit at farm. In the second stage totally postmortem evaluation of 1976 lungs was performed. The assessment of enzootic pneumonia like lesions (EP-like) was made according to Madec and Kobisch (1982) method (1) with own modification, were the presence of scars after previous *M. hyopneumoniae* infections was recorded and pleurisy, pleuropneumonia lesions (PP-like) according to SPES method (2).

Results and discussion

On the basis of survey outcome presence of 3 to 8 pathogens was found on each farm. Infections of *M. hyopneumoniae* were declared most often (22 farms) while *A. pleuropneumoniae* occurrence was reported on 6 farms. *P.multocida* and *H. parasuis* were the least frequently found (3 farms). Respiratory symptoms were observed on 21 farms, the highest intensity of these symptoms was observed in fattening sectors. Comparing own results with those reported in other studies (3, 4) the same trend of polyethiological nature of respiratory disease in pigs was confirmed.

EP-like lesions were observed in pigs from all evaluated farms, number of affected lungs in the different herds range from 37 to 94.7% with a mean score 3.38, while among lungs with lesions the mean score was 4.6. Similar results regarding the high prevalence of EP-like lesions were reported in other countries (5, 6). PP-like lesions were observed in 21 farms, number of affected lungs in the different herds range from 2.4 to 43.2% with a mean score 0.24, while among lungs with lesions the mean score was 1.96. Higher results were reported in Spain and Italy (7, 8). This may result from lower number of fatteners at farms in own study as well as lower concentration of pig production in evaluated region.

Conclusion

Data analysis from farm questionnaires allowed to identify the health risk factors which were in agreement with post-slaughter evaluation. Nonetheless some discrepancies between owner's declaration and evaluation results also were found. The right training, the breeder's trust and commitment will avoid mistakes and first of all allow to rely on the farm questionnaire and postmortem evaluation to introduce some changes in prophylactic programme. The survey must consist of simple and clear questions that farmers understand.

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Effect of iron dextran or gleptoferron supplementation in suckling piglets on preweaning hemoglobin concentration in large-scale commercial farm

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Introduction

Early postnatal iron deficiency anemia in piglets (IDA) is one of the most prevalent deficiency affecting swine husbandry worldwide (1). Piglets are born with little iron reserve and sows milk provides approximately half of piglets requirements only. In addition, modern pig farming has irreversibly eliminated the possibility of natural iron intake from other sources (soil) and created the need for preventive supplementation. Without it, piglets develop IDA rapidly (2). The disease is characterized by paleness and unthriftiness.

The objective of this study was to compare hemoglobin (Hb) concentration (g/dL) between 5 different, routinely applied, commercial iron supplements, taking into account sampling dates and piglet sizes.

Materials and Methods

The study was conducted in high performing sow herd located in Northern Poland, where 8000 sows were separated into 2 equal twin farms. Piglets born from one side were allocated to 4 experimental groups (12 000 animals per each) on the basis of iron product used: Baycox Iron (Bayer), Uniferon (Vetoquinol Biowet), Previron (HIPRA), Gleptosil (CEVA). Piglets born in opposite side were receiving Ferran 200 (VetAgro) during whole trial as a control group.

Intramuscular administration of each product (200 mg Fe equivalent) took place at day 3 after birth. In each group (at day 25 after birth) blood was collected form 3 piglets in every out of 15 randomly selected litters. Before sampling piglets were categorized as small (S), normal (N) and big (B), <6, 6 and >6 kg living of weight, respectively (table 1).

Table 1: Sampling protocol.

Date	Product	Piglet Size
12/2019	Baycox Iron	S= 15; N=15; B=15
	Ferran 200	S= 15; N=15; B=15
02/2020	Uniferon	S= 15; N=15; B=15
	Ferran 200	S= 15; N=15; B=15
03/2020	Previron	S= 15; N=15; B=15
	Ferran 200	S= 15; N=15; B=15
04/2020	Gleptosil	S= 15; N=15; B=15
	Ferran 200	S= 15; N=15; B=15

Control group was sampled the same day. Totally, 360 blood samples were collected by the same swine veterinarian from marginal ear vein after puncture with disposable needle. Hb concentration was analyzed on farm using HemoCue 201+ portable device.

The associations between Hb concentration, product, piglet size and season were determined by using ANOVA linear regression model. The significance level was set at $P<0.05$.

Results and Discussion

In this database, differences in Hb concentration between products were found. Observed parameter was significantly higher after using Previron and Gleptosil (table 2).

Table 2: Preweaning Hb concentration (significant differences in bold).

Date	Product	Big	Normal	Small	Average
12/2019	Baycox Iron	10,69	10,26	10,41	10,45
	Ferran 200	9,99	10,62	10,38	10,33
02/2020	Uniferon	9,45	9,8	9,77	9,67
	Ferran 200	9,67	9,67	9,18	9,51
03/2020	**Previron**	**10,03**	**10,2**	**10,12**	**10,11**
	Ferran 200	8,83	9,13	9,57	9,18
04/2020	**Gleptosil**	**10,59**	**9,45**	**10,94**	**10,33**
	Ferran 200	9,56	10,41	9,39	9,79

Differences between Hb concentration and sampling period were also noticed. Hb level was significantly higher in December than in February and March. When all products were weighted by sampling month (as all were sampled in April) through a statistical model, it was concluded that Hb concentration in piglets supplemented with Previron was significantly higher than with other products. Surprisingly, there was no statistically significant differences between piglets size.

Conclusions

From the results of this study we can conclude that some commercial iron products might be more effective. Piglets after Previron (+10,1%) or Gleptosil (+5,5%) intramuscular supplementation have significantly higher Hb concentration in preweaning age. Additionally, comparison of the results obtained in specific months indicated seasonality. However, factors leading to different Hb concentration on the timeline are unknown. Detailed role of this variation requires further investigation.

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Efficacy of Improvac® to improve back fat thickness of Japanese Kurobuta male pigs

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Introduction

Physical castration of male piglets is a common practice to prevent boar taint in Japan. Physical castrated male pigs tend to have thicker back fat than female pigs (1). Kurobuta is a branded Japanese pork created with Japanese traditional pigs and Berkshire breed. Kurobuta meat is known as high quality but it's carcass's back fat tends to be too thick (2). Carcass weight and back fat thickness are the first check point for carcass quality under Japanese grading system. Immunization against GnRH is an alternative method to physical castration which is expected to result in improved back fat thickness (reduction) by maintaining the male as intact boar for a longer time. In this first study using Kurobuta pigs in Japan, we investigated the effectiveness of immunological castration reducing back fat thickness of Kurobuta carcass.

Materials and Methods

Seven hundred and two pigs with 8 weeks of age were administered with Improvac® (Zoetis Japan Inc.) as the initial injection, and the second injection at about 4 weeks before shipping to slaughter at 8 to 9 months of age. In the control group, 804 piglets were castrated physically within 1 week of age. At the slaughterhouse, their carcasses were graded by Japan Meat Grading Association (JMGA) and carcass weights and back fat thickness were recorded.

Results

Mean carcass weight and back fat thickness are shown in Table 1. Carcass weights of Improvac® group were statistically heavier than the control group (a,b: $P<0.01$), nevertheless back fat thicknesses were significantly reduced (c,d: $P<0.01$).

Table 1. Mean carcass weight and back fat thickness.

	Carcass weight (kg)	Back fat thickness (mm)
Improvac® (n=702)	77.1[a]	22.1[c]
Control (n=804)	72.5[b]	23.3[d]

Student's t-test was used to compare two groups. ab, cd: $P<0.01$.

Approximation straight line between carcass weight and back fat thickness is shown in Figure 1. Improvac® line was always below the line from the control (physical castrated) group and inclination was also moderate suggesting a lower slope. Area surrounded by double line is the range of "Excellent" rank according to JMGA grading. In the Improvac® group, carcasses about 75kg or more are included in the excellent grading grid e.

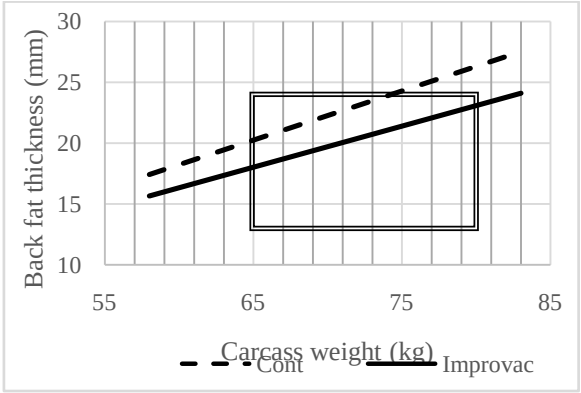

Figure 1. Approximation straight line between carcass weight and back fat thickness

Conclusions and Discussion

Carcass weights from Improvac® administered pigs were statistically heavier than the pigs in the control group (physically castrated). Pork meat from Improvac® pigs tended to be leaner (3). These pigs appeared more slender but muscular than pigs in the control group. Therefore, when the producers estimated appropriate shipping timing in appearance, their weights were already heavy.

Despite the above, back fat thickness in the Improvac® group was thinner than in the control group. Particularly from Figure 1, even carcass weights became heavier, back fat was reduced. From this, if carcasses are heavy, the rank of carcasses could get improved as back fat thinner.

In conclusion, Improvac® is an effective immunological tool for profit management improving the carcass grade of Kurobuta pork comparing to physically castrated pork while effectively avoiding boar taint.

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Metabolomic Comparison of Processing Fluids and Serum from Healthy Piglets

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Introduction

The discovery of new diagnostic biomarkers of viral, bacterial and parasitic infections can benefit the diagnosis, control and eradication of infectious agents. Metabolomic analyses of serum, body secretions and tissues are increasingly employed to identify the interactions between hosts and pathogens, in both human and veterinary research (1-2). Processing fluids from pig production, including serosanguineous transudate from tail docking and testicle fluid from castration of piglets, have been used for microbiological diagnosis of infection in the herd level (3-4), but have not been used as the materials for identifying novel metabolite markers of infections due to the limited understanding on their chemical composition. Therefore, the present study used the LC-MS analysis, multivariate modeling, and metabolite characterization to determine whether tail fluid and testicle fluid contain unique metabolomic profile in comparison with serum.

Materials and methods

Processing fluids and serum samples were obtained from a sow farm negative to *M. hyopneumoniae*, PEDV and PPRSV and separately analyzed by gender.

Both, serum and the two components of processing fluids, testicles and tails, were individually collected. For males, testicle fluid samples (n=10: 2 males from each of 5 different litters), serum samples (n=9, from the same males from which testicles were collected) and tail fluid (pool of 5 tails from other males in each litter) were collected. For females, serum samples (n=9, 2 females from each of 5 different litters) and tail fluid (pool of 5 tails from other females in each litter) were collected.

Serum, testicle fluid and tail fluid samples were analyzed using a liquid chromatography-mass spectroscopy (LC-MS) based metabolomics platform, which included fluid preparation, chemical derivatization (both dansyl-chloride-based and 2-hydrazine quinoline-based derivation), data deconvolution processing and multivariate analysis (MDA), followed by untargeted marker characterization and quantification.

Results

Based on the quantitative analysis, serum, testicle fluid and tail fluid contained significant amounts of free amino acids (AA). Regarding the relative abundance of AA, testicle fluid and serum shared similar profiles, with the same five most abundant AA in different order (Table 1). No statistically significant differences by gender, neither in serum nor in tail fluid were detected. Based on multivariate analysis, testicle fluid and serum samples showed significantly different metabolite profiles, driven by differences in the free AA and complex lipids profile.

Table 1. Five most abundant free amino acids per sample type by descending order.

Sample type	5 most abundant free amino acids
Testicle Fluid	Gly, Pro, Glu, Ala, Gln
Tail Fluid	Ser, Glu, Gly, Ala, Pro
Serum	Pro, Gly, Ala, Gln, Glu.

Discussion

The study results suggest that the metabolomic profile of tail fluid is readily differentiable from serum and testicle fluid, the latter two sharing a similar profile, but different total and relative abundance. The identification of those profiles might help at setting baseline values when performing studies attempting to identify the metabolic changes associated with infections (5).

Furthermore, the data suggests that processing fluids are metabolically rich substrates and they could eventually represent an adequate niche for bacteria to thrive, as their nutritional requirements for maintenance would be fulfilled.

These results justify additional research on the metabolic changes associated with common infections at the herd level and to characterize the host responses to specific infectious agents.

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Detection and characterization of microbial flora and host metabolites in synovial fluids of pigs with clinical signs of lameness

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Introduction

The existence of microbiota in joints of humans suffering arthritis has been confirmed, suggesting a potential correlation with degree of disease (1). Lameness in pigs occurs at different production ages and is associated with an array of risk factors that may differ between production systems. To date, the potential role of inherent microbial flora in pig joints has not been evaluated in understanding lameness development. Thus, this study sought to characterize the synovial microbiota and host metabolic changes in joints of pigs with clinical signs of lameness.

Materials and Methods

Five wean-to-finish farms reporting recent history of lameness were enrolled in the study. Three pigs from each farm were selected based on lameness scores (2): one healthy (score 0) and two lame pigs (score ≥3). Synovial fluid (SF) samples were obtained from eight joints per pig (n=128). Due to low-biomass composition, only 21 SF samples passed the quality control to perform the 16S rRNA sequencing to characterize the microbiota in pig joints. However, the SF from all joints were analyzed for determining host metabolite composition using liquid chromatography-mass spectrometry (LC-MS).

Results

Relative abundance of major phyla in SF samples is shown in Figure 1. The dominant phyla were Firmicutes (dark blue), Actinobacteria (red), Bacteroidetes (grey) and Proteobacteria (purple). Abundance of erysipelas and genus from the family of *Pasteurellaceae* were observed in 57% and 43% of the analyzed SF samples, respectively. These taxa could potentially include bacteria associated with lameness such as *Glaesserella* (formerly *Haemophilus*) *parasuis* and *Actinobacillus suis*. Metabolomics analysis revealed that within each farm, there was a significant difference in amino acid composition profiles in SF between the healthy and lame pigs. The altered amino acid biomarkers identified in each farm were found to be associated with the Krebs cycle as well as amino acid metabolism pathways, indicating increased protein catabolism in the affected joints.

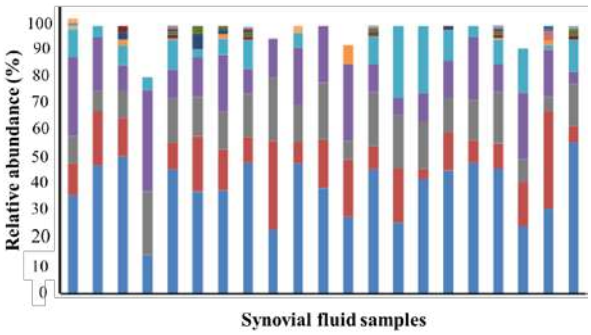

Figure 1. Relative abundance (%) of major phyla in SF samples. Different colors represent different phyla within a sample (column).

Conclusions and Discussion

To date, no literature has analyzed intra-articular bacterial nucleic acids in pigs using high-throughput techniques. Overall, this study suggests existence of bacterial nucleic acids in SF samples from both healthy and lame pigs. The potential association between joint microbiota and lameness remains uncertain. Besides, lameness may result on metabolomic changes in SF from pigs of the same farm.

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Comparing sampling strategies for diagnosis of Senecavirus A from asymptomatic animals

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Introduction

Senecavirus A (SVA) is a non-enveloped, single stranded RNA virus, belonging to the *Picornaviridae* family and has been associated with cases of vesicular diseases in pigs[1]. Previous studies have demonstrated the presence of virus in the tonsils of animals naturally and experimentally exposed to SVA after recovering from clinical signs. These findings led to the possibility of SVA inducing asymptomatic carrier state. The goal of this study was to evaluate and compare different strategies to detect SVA in the acute phase of disease infection as well as during later stages of SVA infection.

Materials and Methods

A total of 28 three-week-old piglets were divided into three groups: Group H, inoculated with an historical isolate (n=12); Group C, inoculated with a contemporary isolate (n=12); and a non-inoculated control group (n=4). Sampling methods included oral, fecal and tonsil swabs, as well as tonsil scrapings, sera collection, and oral fluids. Samples were tested by SVA RT-qPCR, and serological testing was done by indirect immunofluorescence (IFA). The timepoints for collections were on 1, 3, 7, 10, 14, 21, 28, 35, 42, and 48 days post-inoculation (dpi). The percentage of pigs with positive detection of SVA per dpi in each sample type was compared by Kaplan-Meier survival curves (Fig 1 and 2), with the time-to-event defined as the day of a negative PCR result after the last positive detection. The time when the percentage of positive pigs reached 0% is defined as time to negativity.

Results

Oral swabs had an earlier time to negativity from both infected groups, at 28 and 35 dpi, respectively. Fecal and tonsil swabs became negative at 35 dpi in group H and 42 dpi in group C. However, all pigs from both groups had positive fecal swabs up until 21 dpi. Tonsil scraping had a 58% positive detection at 48 dpi in group H, and 17% in group C. Oral fluids from both groups were yielded positive PCR results from 1 to 35 dpi.

Conclusions and Discussion

Overall, oral swabs appeared to be less effective at detecting SVA RNA in inoculated pigs from both groups over time, followed by tonsil swabs and fecal swabs. Due to the reduced number of animals, the point estimates in the survival curves had wide and overlapping 95% confidence intervals, thus no statistically significant difference was found when comparing the proportion of positive detections, except when comparing to a sample type that had reached negativity. Interestingly, tonsil scraping was the only sample-type able to detect positive pigs after 28 and 35 dpi in groups H and C respectively, up until the last day of this evaluation in both groups. Tonsil scraping has shown to be a promising method to detect SVA RNA in the tonsils of asymptomatic animals during the late-phase infection, and oral fluids can also be used to detect SVA in a group level.

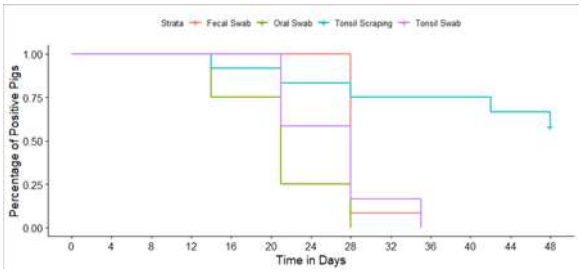

Figure 1. Time to negative detection of historical SVA strain by RT-qPCR based on sample-type.

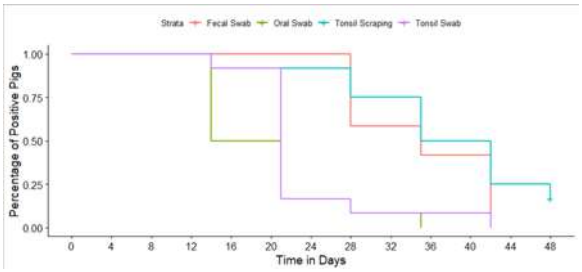

Figure 2. Time to negative detection of contemporary SVA strain by RT-qPCR based on sample-type.

Acknowledgments

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Findings of respiratory diseases of free range wild boar (*Sus scrofa*) subpopulations hunted in the State of Santa Catarina, Brazil

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Introduction

Porcine respiratory disease complex is a multifactorial respiratory syndrome related to the infection with different pathogens, that produces significant losses in swine production worldwide (1). Besides these agents, the occurrence of pulmonary parasites of the genus *Metastrongylus* spp. causes major respiratory tract disease and is associated with the severity of certain respiratory pathologies in domestic and wild boars (2,3). This work aimed to evaluate the findings of respiratory diseases of hunted wild boars in the state of Santa Catarina in the south of Brazil.

Materials and Methods

It was used samples from 59 wild boars hunted between October 2017 and November 2018. Sampling was obtained by convenience, according to the availability of hunters and/or authorization of landowners without observing biostatistics criteria.

The macroscopic findings from the necropsies was documented by photographic register and described in necropsy files. For histopathological evaluation, was collected fragments of tissues, fixed in 10% buffered formalin. The tissues fragments were: cephalic (submandibular and retropharyngeal) and mediastinal lymph nodes; tonsils; lung; liver; spleen.

Results

In the necropsies, the main lesions found were in the lungs, characterized by multiple firm and elevated areas, especially in the caudal lobes, with adult pulmonary parasites compatible with *Metastrongylus* spp. in the lumen of bronchi and bronchioles (25/59, 42,4%). One animal presented pulmonary hepatization (1,7%) compatible with bacterial bronchopneumonia, and in another animal, it was observed multifocal lobular consolidation with chessboard aspect (1,7%), suggestive of influenza. Granulomas with caseous and/or mineralized lesions compatible with tuberculosis were not detected in the lungs, diaphragmatic arch, liver, spleen and lymph nodes.

In the histopathological evaluation, the most affected organ was the lung (46/59, 76,7%), and the results are summarized in Table 1. The most frequent injury patterns were: mixed inflammatory infiltrate interstitial pneumonia; bronchopneumonia with predominance of eosinophils; moderate bronchi-associated lymphoid tissue hyperplasia (BALT); and multifocal granulomatous pneumonia, characterized by the presence of epithelioid macrophages and multinucleated giant cells. These changes were, in 54,4% of cases (25/46), associated with the intralesional presence of adult worms and/or free larvae in the pulmonary parenchyma.

Table 1. Frequency of histopathological findings in wild boars

Lung lesions	N total	Positive frequency	%
Interstitial pneumonia	59	35	59.3
Eosinophilic bronchopneumonia	59	24	40.7
BALT hiperplasia	59	24	40.7
Granulomatous pneumonia	59	6	10.1

Conclusions and Discussion

The prevalence of *Metastrongylus* spp. suggests that most of the microscopic lesions observed in the lung are related to pulmonary parasitism because the presence of eosinophils in moderate or large amounts was observed in most cases and the intralesional presence of the parasite was observed as well. Even if the presence of *Metastrongylus* spp. in wild boars is relatively common, the risk of being associated with infections in conjunction with other diseases, such as tuberculosis and circovirosis should be considered.

A wild boar presented lung with multiple reddish areas of consolidation in the middle lobes, with chessboard aspect, being the lesion suggestive of influenza. In contrast to commercial pigs, wild boars have the opportunity to contact other wildlife, wild birds, domestic herds and their habitats, and there is a concern that wild boars may have a mixed IAV infection and generate rearrangements of viruses from poultry and swine, which could be transmitted to domestic swine or humans (4). These factors should be considered and further studies should be conducted focusing on this subject.

Acknowledgments

Santa Catarina Integrated Agricultural Development Company, Brazilian Swine and Poultry Agricultural Research Company and Federal Institute Catarinense.

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NUTRITION

Evaluation of MiaTrace Zn as an alternative zinc source in piglet diets

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Introduction

A trial was conducted to evaluate the efficacy of a novel source of Zn (MiaTrace Zn) as an alternative zinc source in the diets for newly weaned piglets compared to both, additive and therapeutic doses of ZnO.

Materials and Methods

A total of 135 newly weaned piglets ([Large White x Landrace] x Pietrain; 5.8 ± 0.92 kg BW; mixed sexes) of 21 days of age were randomly distributed by initial body weight into 9 blocks (3 pens of 5 pigs per block). The experimental treatments consisted of: a negative control (NC) treatment with 120 ppm Zn from ZnO; a positive control (PC) treatment with 2520 ppm Zn from ZnO during the pre-starter phase (therapeutic dose) followed by 120 ppm Zn from ZnO during the starter phase and; a test treatment (MTZ) with 120 ppm Zn from MiaTrace Zn (Table 1). Within each block, the three treatments were randomly distributed among the three pens. The pigs were individually weighed at the start of the trial and at days 14 and 42. Feed intake between each weighing interval was also recorded for each pen. Fecal score (for each pen) was also assessed daily using a 5-category score system (0=firm and shaped; 1=soft and shaped; 2=soft without shape; 3=loose; 4=watery). Data were analyzed using the GLM procedure of the statistical package SAS. Differences with a P-value of less than 0.05 were considered significant.

Table 1. Experimental treatments and the amount of Zn (mg/kg of feed) provided by the different products in the pre-starter (PS) and starter (ST) phases.

	ZnO		MiaTrace Zn	
Treatment	PST	ST	PST	ST
NC	120	120	-	-
PC	2520	120	-	-
MTZ	-	-	120	120

Results

No statistically significant effects of dietary treatment on performance were observed during the pre-starter phase ($P > 0.05$). However, during the starter phase and the overall experiment, the therapeutic dose of ZnO significantly increased ($P < 0.05$) body weight gain and feed intake relative to NC. The piglets offered MTZ also presented an increased feed intake ($P < 0.05$) relative to NC during the starter phase and the overall experiment. No differences were observed for any parameter between PC and MTZ in any of the periods considered (Table 2).

Table 2. Average performance of piglets between 0-42 days of trial.

Treatment	Weight gain (g/d)	Feed intake (g/d)
NC	366 b	481 b
PC	393 a	516 a
MTZ	387 ab	516 a

ab Values in the same column with different letters are significantly different ($P<0.05$)

Generally, the animals presented good health and no diarrhea was observed. Fecal scores were very low and no statistically significant effects of dietary treatment were observed among treatments in any of the experimental periods considered ($P > 0.05$).

Conclusions and Discussion

It is concluded that, under the conditions of the current trial, the use of therapeutic doses of ZnO during the pre-starter phase significantly improved feed intake and weight gain over the whole experimental period. In addition the replacement of the conventional dose of ZnO with MiaTrace Zn also improved feed intake significantly, and no differences were observed between the therapeutic use of ZnO and MiaTrace Zn.

Effects of polyphenols (hydroxytyrosol and carnosic acid) supplementation on IUGR frequency occurrence in swine

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Introduction

New genetics are commonly based on a higher prolificacy selection criterion and have been established in intensive production due to their greater profitability. This fact compromises adequate fetal development, due to competition between littermates for the limited space available in the uterus for implantation and inappropriate placental development among other factors (1) resulting in intrauterine growth restriction (IUGR) which represents a physiopathological condition in which a fetus cannot reach its genetically determined size (2). Pigs suffer the highest rate of embryonic loss (up to 50%) and the most severe natural intrauterine growth restriction (3,4). Oxidative stress is related to certain pregnancy disorders such as abortion, IUGR and prenatal mortality (5,6). The present study aimed to analyze the usefulness of hydroxytyrosol and carnosic acid supplementation as alternative antioxidant sources during gestation in the frequency of IUGR occurrence in the offspring of sows in commercial herds.

Materials and Methods

A total of 23 female breeding pigs from the 1st to the 7th litter were allocated under the same conditions simultaneously into two treatment groups during the whole gestation period to compare the effects of supplementation in the feed with hydroxytyrosol and carnosic acid (MiaPhenol; 150 mg/kg feed; total phenol content 35 mg/g MiaPhenol; group MPH), remaining a control group untreated (CG). Both groups received the same basal diet formulated to meet the requirements during pregnancy (NRC, 2012), fed once a day, while having *ad libitum* access to water. The daily amount of feed supplemented during the entire gestation phase was 2.5 kg per animal and day. Data from 418 piglets of which 243 were from the MPH group while 175 were from CG. In all the piglets, body weight and size (biparietal diameter, abdominal and thoracic circumferences, occipito-nasal and occipito-caudal lengths, obtained with caliper gauge and a measuring tape) were recorded individually at the farrowing moment (<12 h post farrowing). To classify animals as IUGR, the average live weight of each treatment was calculated and a standard deviation (IUGR1sd) or two (IUGR2sd) were subtracted. Data were analyzed using a statistical package SPSS v. 15.0. Comparisons of means were made using a Student's t-test. Significant differences of $p<0.05$ were always considered significant.

Results

In MPH group, both, number of live-born piglets per litter (16.63±0,22 vs. 16.31±0.17 ; P=0.53) and live weight at birth of the piglets (1.33±0.02 vs. 1.29±0.02 Kg; P=0.54) were numerically higher compared to the CG. Biparietal diameter (4.65±0.17 vs. 4.58±0.19; P=0.01) and thoracic circumference (24.03±0.14 vs. 23.58±0.18; P=0.045) were statistically higher in MPH group piglets. Abdominal circumference (19.67±0.14 vs. 19.37±0.21; P=0.21), occipito-nasal length (9.57±0.04 vs. 9.57±0.06; P=0.18) and occipito-caudal length (25.10±0.14 vs. 24.95±0.18; P=0.51) did not show statistical differences, but with a trend to be numerically higher for MPH group. IUGR1sd percentage was similar (16.9 vs. 16.6 %) while its IUGR2sd in MPH was lower than in the CG (2.9 vs. 3.4%).

Conclusions and Discussion

The present trial indicates that although the supplementation with hydroxytyrosol and carnosic acid during gestation period increased live-born piglets per litter and its sizes and live weight at birth, did not vary the frequency of IUGR piglets while applying 1 or 2 standard deviation. So, this study suggests that the addition of polyphenols could result in a slight increase of live-born piglets per litter and their live weight at birth without a negative impact in IUGR appearance. Further studies are needed to clarify mode of action and efficacy of MiaPhenol.

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Polyphenols (hydroxytyrosol and carnosic acid) supplementation impact on late gestation metabolic status and lactation body mobilization of sows

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Introduction

Oxidative stress occurs during reproduction in the sow. Increased oxidative stress was reported to be an important factor causing decreased availability of antioxidants during late gestation, which could impair placenta and fetal growth (1) as well as negatively affects the well-being and health status of sows (2). It is a fact that oxidation rate increases as metabolism rate elevates. In this way, among other shown consequences of oxidative stress, further complications of diabetes mellitus are described (3). Likewise, high lactational weight loss drives to a severe oxidative stress and metabolic damage (4). Additionally, excessive weight loss in sows negatively influences weaning to estrus interval, subsequent farrowing rates, total born litter sizes and piglets birth weight (5). The aim of the present study was to analyze the usefulness of hydroxytyrosol and carnosic acid as alternative antioxidant sources during gestation supplementation on negative late gestion metabolic status and high lactation body mobilization of sows in commercial herds.

Materials and Methods

The experiment was performed on 79 sows (1-7 parities) divided simultaneously under the same conditions into two groups during the whole gestation period to compare the effects of supplementation in the feed with hydroxytyrosol and carnosic acid (MiaPhenol; 150 mg/kg feed; total phenol content 35 mg/g MiaPhenol; group MPH), remaining a control group untreated (CG). Both groups received the same basal diet formulated to meet the requirements during pregnancy (NRC, 2012), fed once a day, while having *ad libitum* access to water. The daily amount of feed supplemented during the entire gestation phase was 2.5 kg per animal and day. Visual body condition (BC1 and BC2), as well as, backfat (BF1 and BF2) and lean muscle mass (LMM1 and LMM2) were measured by ultrasounds. Each sow was scored on day 110 and at weaning moment. A percentage of randomly selected sows blood samples (MPH=15; CG=10) were drawn on day 110 of gestation to assess parameters of glucidic metabolism (glucose, fructosamine) and lipid metabolism (triglycerides and total-, HDL- and LDL-cholesterol).

Data were analyzed using a statistical package SPSS v. 15.0. Comparisons of means were made using a Student's t-test. Significant differences of $p<0.05$ were always considered significant. Differences for body composition parameters were assessed by ANOVA of correlation covariation with parity of sows and days between measurements.

Results

MPH group shows a significantly higher BC1 (3.20±0.17 vs. 3.02±0.10; P=0.03) and BF1 (13.85±0.93 vs. 12.34±0.68; P=0.001), while significantly lower LMM2 (45.33±1.57 vs. 46.16±1.17; P=0.01). BC2 (2.97±0.10 vs. 2.87±0.08; P=0.55), BF2 (10.9±0.92 vs. 10.8±0.59; P=0.05) and LMM1 (47.32±1.36 vs. 48.46±0.98; P=0.05) did not differ statistically among groups. During lactation period, MPH group mobilized more BF and less LMM compared to CG (2.86 vs. 1.59; P=0.19; 1.85 vs. 2.06; P=0.28; respectively). There were no differences comparing biochemical blood data between groups (Table 2).

Table 2. Biochemical blood parameters

(mg/dl)	MPH	CG	p-value
Glucosa	77.40	82.60	0.28
Fructosamine	331.73	336.80	0.60
Triglycerides	64.66	70.40	0.44
Cholesterol	62.00	62.36	0.32
HDL	23.96	22.69	0.44
LDL	38.18	36.34	0.47

Conclusions and Discussion

The present trial indicates that the supplementation with hydroxytyrosol and carnosic acid during gestation period enhance sows body condition and back fat reserves before farrowing, without affecing metabolic status. So, this study suggests that the addition of polyphenols could result in a selected back fat mobilization remaining lean muscle mass during lactation. Further studies are needed to clarify mode of action and efficacy of MiaPhenol.

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Impact of a phytogenic feed additive in newly weaned piglets experimentally challenged with *Escherichia coli*

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Introduction

Weaning is a challenging and critical time in the piglets' life due to changes in diet, environment and bacterial challenges. These challenges often lead to post weaning diarrhea (PWD) in which *Escherichia coli* is one of the most common pathogens. To control PWD, antibiotics (AB) and ZnO are often used during the post weaning period. Due to the potential involvement in the development of antimicrobial resistances and environmental impact, it is important to look for alternatives for both AB and ZnO. The objective of this study was to evaluate the impact of a phytogenic feed additive (Fresta® Protect) on zootechnical performance and resilience of newly weaned piglets challenged with an F18 *E. coli* strain.

Materials and Methods

Thirty-six healthy piglets (LW × LR) confirmed negative for haemolytic *E. coli* but genetically susceptible to F18 *E. coli* with an average age of 30 ± 1 days were included in this trial.

Piglets were allotted to three experimental groups based on body weight and gender, housed individually in pens with plastic slatted floor. A summary of the different treatments is shown in table 1.

Table 1. Summary of the different treatments

Group	Treatments
T1	Infected and treated (IT): basal diet (BD) + Fresta® Protect 1000 mg/kg
T2	Infected, not treated (INT) (BD)
T3	Not infected, not treated (NINT) (BD)

Individual feed intake was recorded daily (ADFI). Piglets were individually weighed and average daily gain (ADG) was calculated. Piglets were clinically examined individually during the whole trial, this included observation of faecal consistency (score: 0 = normal to 3 = severe diarrhoea) and colour. Individual faecal samples were collected, on d-1 and from d1 to d4 for microbiological analysis. At d7 of the trial, 4 piglets / group were euthanised and tissue samples were harvested for observation and morphology analysis. At d14 the remaining animals were euthanised and sampled.

Results

No significant difference was observed in the body weights achieved at the end of the trial for the different treatment groups. However, the difference on ADG and ADFI in T2 and T3 show clearly the efficacy of the challenge model. Although T1 and T2 showed similar feed intake, piglets in T1 numerically showed higher ADG than T2 during d 4 – d 7 (see table 2).

Table 2. Summary of the ADG results

	D 0 - 4 (g/ d)	D 4 - 7 (g / d)
T1	224.3	204.3^{ab}
T2	256.3	98.9^{a}
T3	377.9	415.2^{b}

Different superscripts indicate statistical significance ($P < 0.05$)

By d 2 - 4 faecal shedding of HEC was significantly lower ($P < 0.05$) in T1 than in T2.

No significant differences were observed for mortality during the trial. A summary of the faecal total bacterial counts, HEC, HEC /total bacterial count (log_{10} CFU/g) may be seen on table 3.

Table 3. Summary of microbiological analysis from d-1 to d4.

	Total count	HEC	HEC/total
T1	8.10 ± 1.22	1.34 ± 2.75^{a}	0.19 ± 0.37^{a}
T2	7.65 ± 1.02	3.08 ± 3.52^{b}	0.40 ± 0.46^{b}
T3	7.47 ± 1.26	0	0

Different superscripts indicate statistical significance ($P < 0.05$)

Tissue analysis showed less fusion of villi ($P < 0.05$) in T1 than in T2, and less follicular hyperplasia in T1 than in T2 ($P<0.05$). A summary of the histological assessment may be seen on table 4.

Table 4. Summary of the histological assessments at d7 and d14 of trial

	Fusion of the villi	Follicular hyperplasia
T1	1.29 ± 1.25^{a}	0.29 ± 0.76^{a}
T2	2.00 ± 0.89^{b}	0.83 ± 1.33^{b}
T3	na	na

Different superscripts indicate statistical significance ($P < 0.05$)

Conclusions and Discussion

Dietary inclusion of Fresta® Protect in the conditions of this trial improved growth performance of piglets compared to the challenged control group. It also resulted in significant reduction of both *E. coli* shedding and histological alterations. Therefore Fresta® Protect could be considered a suitable candidate in order to sustain good growth and increase resilience against *E. coli* induced PWD post weaning.

Effect of a phytogenic feed additive on intestinal fermentation patterns and intestinal barrier integrity in piglets

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Introduction

Weaning in commercial pig farms is carried out earlier than in natural pig communities (10 to 12 weeks of age) (1), this subjects the piglets to dramatic changes in feed composition, management and immunity that the piglet is yet not prepared for. The intestine suffers changes to its structure and function, epithelial barrier function is compromised in the immediate post-weaning period, where weaning causes a more permeable small intestine, which may lead to translocation of toxins, allergens, viruses or even bacteria (2).

The objective of this trial was to assess the impact of a phytogenic feed additive, Fresta® Protect on zootechnical performance, intestinal fermentation and intestinal barrier integrity of piglets post-weaning.

Materials and Methods

120 piglets of 24-25 days of age and approximately 6.26 kg of liveweight were allocated according to body weight and gender to two treatment groups. A summary of the trial design is shown on table 1.

Table 1. Dietary treatments applied to post-weaning piglets

	Treatment
T1	Control (NC) – Basal diet (BD)
T2	BD + 1000 mg/kg Fresta® Protect

The piglets were fed two diets throughout the duration of the trial, pre-starter (day 0 to 14) and starter (day 14 to 42) diets. Piglets were weighed individually (BW) at days 0, 14, 28 and 42, average daily feed intake (ADFI) was measured, average daily gain (ADG) and feed conversion ratio (FCR) were calculated. Faeces were visually assessed and scored. On days 14 and 42, one piglet per pen was euthanised for sampling and gut barrier integrity was measured by ex vivo FITC-4kDa permeability assay in distal small intestine samples. Short-chain fatty acids and lactic acid analysis was quantified in the intestine (96 samples, d14) as well as tight junction protein expression. Any deaths or treatments were recorded and mortality (%) was calculated. Data were analyzed by mixed procedure using the SAS statistical software, differences were considered significant at $P < 0.05$.

Results

No significant difference was found on the final piglets body weight between T1 and T2 (19.6 and 20.2 kg). From d14 – 42, there was a tendency for a faster growth of pigs of T2 than T1. This may be explained by higher ADFI in T2 than T1 (897 vs 844 g/d; P=0.069)). This resulted in faster growth from d0-42 for T2 than T1 (437 and 414 g/d; P=0.058). No significant differences were observed for FCR, mortality or veterinary treatments, diarrhoea incidence, faecal scores for both treatments. Stomach acidity showed a trend for lower pH in T2 than in T1 (3.6 and 3.9). Expression of the tight junction proteins ocludin and Zo-1 was numerically higher in T2 than in T1 (1.21 and 0.98 1.6 and 1.47, respectively). Fermentation patterns and lactate content in the mid colon were also altered (table 2).

Table 2. Summary of the fermentation patterns and lactate content in mid colon

	T1	T2
Short-chain fatty acids, µmol/g	119	130
Acetate, %	59	55.9
Proprionate, %	24.2	25.3
Butyrate, %	12.8	13.4
Iso-butyrate, %	0.69	0.67
Valerate, %	3.2	3.9
Iso-valerate, %	0.82	0.79
Lactate, µmol/g	11.3	13.8

The assessment of gut barrier integrity demonstrated that small intestine permeability was reduced by 69.3% in T2-Fresta® Protect fed piglets compared to T1 (P=0.049), (3).

Conclusions and Discussion

In the conditions of this trial, the lower stomach pH in T2 of 0.3 points can be considered relevant as lower pH leads to increased barrier function and improved protein hydrolysis and mineral solubilization. Altered fermentation patterns between animals offered Fresta® Protect and T1 demonstrate the beneficial impact of the phytogenic feed additive. The dietary inclusion of Fresta® Protect also resulted in a significant strengthening of the gut barrier integrity.

This trial shows that Fresta® Protect can be a valuable additive in supporting post weaning performance and intestinal integrity.

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Effect of DeviCool® on lactating sow performance in Italian summer conditions

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Introduction

Global warming is causing more extreme weather phenomenon in temperate and Mediterranean climate zones. Genetic selection for increased litter size together with leaner genotypes has produced sows with a lower voluntary feed intake much more prone to suffering from summer heat stress (HS) (1). A threshold of 25°C was sufficient to provoke HS , cause prolonged farrowings and reduced feed intake (2). The influence of high environmental temperature has been shown to lower the sow's performance (3).Data from a large Spanish database has shown a seasonal "high" in piglet mortality and weaning to service interval in the months of August and September.(4)The last decade has seen increases in pig production in typically hot climates such as Asia ,South America and Spain. The need for a nutritional strategy to combat the ever common phenomenon of HS during lactation is urgently needed. Due to the reduced feed intake in periods of heat stress , the lactating rations must be concentrated specially in terms of energy and vitamins.

The objective of this study was to test the effects of a feed additive, DeviCool®, on the sow's performance under HS conditions.

Materials and Methods

120 sows were weighed and backfat(P2) was measured for each sow on entry to the farrowing house and at weaning to record weight and body condition loss. Temperature and humidity were recorded, every thirteen minutes, throughout the whole lactation period. Sows were divided into two groups and balanced for parity and body weight. The control group was fed the standard farm mixed farrowing ration. The treatment group was fed the same ration with the addition of 3kg/ton of DeviCool®, containing agents known to help with vaso dilazione as well as calcium and potassium homeostasis, thus reducing heat production. Individual piglets heat production from seven litters from both the control and the treatment groups were individually identified at birth and weighed at birth and weaning.

Statistical analysis was performed using IBM SPSS version 25,0

Results

The temperatures in the farrowing housed ranged from a minimum of 24,7°C to a maximum of 34,6°C with an average of 27,9 and a relative humidity of 65%. There was a total of over 5.000 temperature recordings. In the control group, 82% of the recordings were >24°C, and in the treatment group 88% of the readings were> 24°C.

No significant difference in weight and backfat(P2) losses were found during lactation between the control and treatment groups. Sows that were offered the DeviCool® treatment had a numerically lower piglet mortality (10,84% vs 14,88%). The difference, however, was not significant.

An increased daily live weight gain was observed in the piglets in the treatment group (Table 1).

Table 1. Piglet daily live weight gain

	Control	Treatment	SEM	Sig
DWG(g/day)	0,189	0,210	0,003	<0,001

Conclusions and Discussion

Metabolizable energy intake is decreased at environmental temperatures above UCT,which can range between 18 and 22°C(5).A decline of 25% in milk yield was observed after four days at 28°C(5).Another study showed 18% lower mean litter daily gain in HS conditions(6).The reduction in piglet daily liveweight gain at temperatues over 25°C(3) were very similar to the control data in this study.Considering more than 80% of the temperature recordings were in eccess of 24°C , we can assume that HS was present in all of the sows.

Modern hyper prolific sows can be in a severe catabolic state and undergo increased oxidative damage during late gestation and lactation(7).In conditions of high oxidative stress,there will be reduced reproductive performance in terms of lactating capability and subsequential weaning to service interval.

The feed additive DeviCool ® has been shown to be effective in increasing the piglet daily live weight gain and tending towards reducing preweaning mortality.

From an economic point of view , the lower mortality and incresed weight of the piglets , under Italian market conditions, produced a return on investment of 6:1.

Acknowledgments

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Effect of DeviCool Plus Liquid® on heavy pig production for Parma Ham in Italian summer conditions

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Introduction

Global warming is causing more extreme weather phenomenon in temperate and Mediterranean climate zones. Genetic selection for increased leaner genotypes has produced pigs with a lower voluntary feed intake much more prone to suffering from summer heat stress. In typical Italian summer conditions, it is common to encounter severely reduced growth rates in heavy finishing pigs in the months of July and August. The annual "high" in market prices is usually found in September and October (1), so the producer loses up to 15kg of live weight per pig, when the prices are high. The objective of this study was to look at the effect of a feed additive, DeviCool Plus Liquid®, on the finishing pig performance.

Materials and Methods

Two typical Po Valley finishing barns were used for the study. The pens were partially slatted and a liquid feeding system was used (with the addition of water only). The study ran from 12 July until the 24 of September 2019. A total of 642 pigs were allocated to the control group and 563 to the treatment group. The pigs in the control group were fed a standard farm mixed finishing ration and the treatment group the same ration with the addition of 5kg/ton of DeviCool Plus Liquid®, containing agents known to help with vaso dilazione as well as calcium and potassium homeostasis, thus reducing heat production .All the pigs were from the same source farm, and had a genetic composition of Topigs Talent x PIC Camborough (PRRS positive). Sixty pigs from each of the two groups were randomly selected and individually tagged. The tagged pigs were weighed at the start of trial, after 56 days and at the end of the trial. Continuous temperature and relative humidity recording were carried out throughout the study. The total feed consumption was recorded for each group. After slaughter, gastric ulcer scoring was performed using the method described by Robertson (2)

Statistical analysis was performed using IBM SPSS version 25,0.

Results

The control pigs had a start weight of 99,6kg ± 13,5 kg and the treated pigs had a start weight of 93,1kg ±11,5kg There were no significant differences in the growth rate between the groups (853g/day vs 851 g/day). The treated pigs had a numerically improved feed conversion rate (FCR) of 3,245 vs 3,358, although this difference was not significant.

Over 5500 temperature recordings were made, with a minimum of 21,1°C and a maximum of 34,1°C. The average was 27,4°C with an average relative humidity of 74%. The treated pigs showed better gastric ulcer scores, with significantly higher numbers of individuals with no lesions and lower numbers showing pre ulcer changes

Table 1

Gastric Ulcer Scores

	score	Control	Treatment	Sig.
No lesions	0	7	23	*p<0,05*
Pre ulcer	1	91	67	*p<0,05*
Slight ulcers	2	10	12	NS
Severe ulcers	3	0	0	NS
Total		108	102	
average		1,03	0,89	

A 2x3 factorial analysis was performed using treatment and three groups based on start weight 70/90kg, 90/110kg and >110kg.The growth rate was calculated at the intermediate and final intervals. The pigs offered DeviCool Plus Liquid in the 70-90 kg start weight group, grew significantly better (*p< 0,05)* in the period to the intermediate weighing and tended to grow better over the full finishing period.(*p<0,08)*.The control group had total losses(mortality and second grade pigs) of 3,4%, while the treated group had total losses of 1,2%

Conclusions and Discussion

The effect of modern genetic to be more susceptible to HS and reduced daily feed intake has been demonstrated (3). The same study also showed the higher susceptibility to heat stress in heavier pigs. Heavier pigs showed reduced feed intake in HS conditions in another study (4)

The overall economic damage of HS to the whole US livestock industry was estimated at $2,4 billion and $299 million to the pig sector (5).

The better gastric ulcer scores in the treated pigs can be attributed to the fact that satiated pigs will be less stressed.

The feed additive DeviCool Plus Liquid ® has shown to be effective in reducing total losses, improving FCR and increasing daily live weight gain amonst the smaller pigs and reducing gastric ulcers.

Acknowledgments

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Fecal pH throughout the reproductive cycle of sows in commercial pig herds

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Introduction

Measuring the pH of feces is a simple and cheap method that can be performed on living animals and that may provide information about intestinal health. Recently, in pig farms suffering from new neonatal piglet diarrhea syndrome (3), very high fecal pH values (>8.5) were observed in many sows of affected piglets (1). However, there are no fecal pH reference values for the current high-performing sow breeds.

The present study investigated the fecal pH of sows from different farms throughout the reproductive cycle along with data on sow body condition, feed composition and coarseness of the feed.

Materials and Methods

Three pig herds with commercial hybrid sows without health problems were included. A general description of the farms is shown in Table 1.

Table 1. General description of the 3 farms (A-C)

	A	B	C
Number of sows	230	350	500
Batch farrowing system	4-wk	3-wk	5-wk
Lactation length (d)	21	28	24
Type of feed:			
Gestation	crumb	pellet	meal
Lactation	pellet	pellet	meal
WII[a]	meal	pellet	meal
Pigs weaned/sow/yr	33	29	40

[a]WII: Weaning to Insemination Interval

In all farms, sows received approximately 3 kg feed per day during gestation. Prior to expected farrowing, the feeding level was decreased to 2.8-3.0 kg/sow/day. After farrowing, the level was gradually increased until maximum levels (7 to 9 kg/day) were reached at around day 10 post-partum. Within each herd, 10 sows were selected and rectal feces samples were collected at different timepoints during the cycle. Upon arrival in the laboratory, the samples were diluted at a ratio 1:3 feces with distilled water as described by Hydock et al. (2). The measurements were done using a calibrated pH measuring device (HI 9125 pH/ORP meter, Hanna Instruments, Temse Belgium). In addition, data on sow body condition (back fat), feed composition and coarseness of the feed were collected.

Results

The fecal pH values in sows from three farms at different timepoints during the reproductive cycle are shown in Table 2.

Table 2. Mean (95% CI) fecal pH in healthy sows of three farms (A-C)

	A	B	C	All farms
D90 G	6.77	7.25	7.31	7.11[a,b] ;6.97-7.25
D1 L	6.90	7.18	7.07	7.05[a] ;6.91-7.18
D3 L	7.15		7.13	7.14[a] ;6.96-7.32
D7 L	6.94	7.11	7.40	7.14[a] ;7.04-7.24
D14 L	6.77	7.02	6.99	6.93[a,b] ;6.81-7.04
D21 L	6.72	6.99	6.98	6.89[b] ;6.77-7.01
D7 PW	6.63	7.28	7.64	7.15[a] ;6.97-7.33
D30 G	7.09	7.48	6.84	7.15[a] ;6.99-7.31
All time-points	6.87	7.18	7.17	7.06 ;7.01-7.11

G gestation L lactation PW post-weaning

The sows in each of the farms lost 2-3 mm backfat during lactation. The backfat levels of sows in herd B were 3-4 mm higher than those of the sows in herds A and C. The protein content of the feed ranged from 14.2 to 18.7%, crude fiber from 5.5 to 7.0%, crude fat from 2.6 to 5.4%, and the mean particle size of the feed from 0.8 to 1.2 mm. The pH of the drinking water was 8.2 (herd A), 7.7 (herd B), and 6.4 (herd C).

Conclusions and Discussion

The fecal pH of individual sows ranged from 6.30 to 7.93. However, for all herds together, the average fecal pH value of healthy sows throughout the reproductive cycle ranged from 6.89 to 7.15. The variations due to sow and time of sampling during the reproductive cycle were low with coefficients of variation of less than 5%. The results showed that in the last stages of lactation (i.e. at day 21), significantly lower average pH values ($p \leq 0.05$) are expected when compared to other stages of the cycle. Bearing its limitations, the study provided reference fecal pH values from high-performing commercial sows under field conditions and as such they could be used directly in the field. Further research is needed to elucidate factors affecting fecal pH values of sows.

Acknowledgments

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Use of acidifiers in the nursery phase as an alternative to antimicrobials swine growth promoters

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Introduction

The current use of antibiotics growth promoters (AGP) is associated with the transmission of resistance genes and residues in meat (5). Several alternative additives to AGP have been tested (2), highlighting prebiotics and organic acids (6,7). In the nursery is where the challenges of AGP removal are most evident, because piglets are subjected to a series of stressors associated with physiological, enzymatic and immune immaturity of the gastrointestinal tract (6). The objective of this study was to evaluate the use of a blend of prebiotics and organic acids against a colistin diet, on performance and diarrhea control in nursery piglets.

Materials and Methods

150 pigs from PIC genetics (castrated males and females) were used, 22 days of average age and 5.568 ± 0.781 kg live weight. Housed in stalls with 2.55m^2 of area. The experimental design was randomized blocks with three treatments and 10 repetitions per treatment, with the five animals stall being the experimental unit. The treatments were: T1 - Negative Control; T2 - Colistin - 10 mg / kg; and T3 - Mannooligosaccharides + Beta-glucan + Ammonium formate, Formic acid, Ammonium propionate and acetic acid (1 kg / ton). The diarrhea score was performed daily (8). Calculation of diarrhea index: Diarrhea Index = number of days with diarrhea / number total days of the test. On the 24th day of housing one animal per stall was randomly selected and euthanized for cecal content collection for quantification of *Lactobacillus* and *E. coli* and total coliforms. Parametric data were subjected to analysis of variance and means to Tukey test, using statistical program R version 3.3.0 (2016-03-05). Nonparametric data were evaluated by Chi-square test. Differences with P 0.05 were considered statistically significant.

Results

Differences in favor of groups T2 and T3 were observed in relation to T1 for daily weight gain (DWG) and feed conversion (FC) in the total evaluation period (Table 1). For diarrhea score 3, group T3 showed intermediate result (P> 0.05), compared to T1 and T2 (Table 2), with the latter showing better effects (P <0.05) compared to T1.

Conclusions and Discussion

As for the FC, the results obtained in the calculation of the whole experimental period were similar where they registered advantages for diets with organic acids (2). The improvement in FC with the use of dietary acidifiers results from the probable better utilization of dietary protein, added to the antimicrobial action of organic acids (1,3). In this sense, our results were similar to studies using organic acids compared to Tiamulin (30 ppm) and also found no differences between treatments (5). The tendency of diarrheal symptoms to improve is in line with findings in various literature (1,2,7). T2 had higher cecal *E. coli* and total coliform counts compared to T1 and T3, while T1 and T3 were similar. This may suggest intestinal microbiota dysbiosis in T2 at the therapeutic dose used. (1). There was no statistical difference for the *Lactobacillus* count between treatments, but T3 favored the reduction of *E. coli* and fecal coliforms in relation to T2, not differing from T1. Nursery phase diets supplemented with a combination of Mannooligosaccharides + betaglucans + acidifiers provide similar performance to colistin supplemented diets and promote good antibiotic stewardship. Based on the results of this study we can conclude that acidifiers are a promising alternative to the growth promoter antimicrobials used in pig farming.

Table 1. Average performance values of piglets submitted to experimental treatments.

	TREATMENTS[1]			
DATA	T1	T2	T3	P value
DWG	0,356	0,375	0,366	0,197
FC	1,679b	1,602a	1,593a	0,070

[1] a,b means followed by distinct letters on the line indicate difference by Tukey test (P <0.05).

Table 2. Occurrence values of diarrheal cases, scores 2, 3 and diarrhea index of piglets submitted to experimental treatments.

	TREATMENTS[1]		
SCORE	T1	T2	T3
2	0	0	0
3	21b	09a	14ab
Diarrhea Index	0,42	0,18	0,28

[1] a,b means followed by distinct letters on the line indicate difference by the chi-square test (P <0.05).

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Conjugated linoleic acid: Effect on pig growth performance from 135 to 176 days of life

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Introduction

Conjugated linoleic acid (CLA) consists of a group of polyunsaturated fatty acid that are positional and geometric (cis or trans) isomers linoleic acid (C18:2). The incorporation of CLA in animal feed is key to obtain higher food quality for human consumption since interest in CLA research has increased in recent years due to different studies that have shown that it is a physiologically active compound, with many potential beneficial effects for human health. On the other hand, there is evidence that CLA improves daily weight gain and nutritional efficiency at increasing inclusion levels (1). The objective of this trial was to evaluate the use of a CLA-based supplement (Lutalin® - Basf, Ludwigshafen, Germany) on growth performance and carcass quality in fattening pig fed diets.

Material and methods

This trial was conducted at Biofarma Research Centre S.A - Argentina. 168 pigs (castrated males and females) distributed in randomized delineation were selected in 2 treatments with 12 pens per treatment and 7 animals per pens. The treatments were: Control without CLA inclusion and Test with the inclusion of 0.50% of CLA-based supplement (Lutalin®-Basf, Germany) in the feed from 135 to 176 days of life. The performance parameters evaluated were: Final weight (FW), average daily feed intake (ADFI), average daily gain (ADG) and feed conversion (FC). All parameters were subjected to an analysis of variance (ANOVA) through INFOSTAT® (Córdoba National University, Argentina) with significant statistical difference $P<0.05$.

Results and Discussion

Final weight, average daily feed intake and average daily gain was not affected by CLA used in the same way as reported by (2) and in contrast to (1,3) who observed improvements in ADG with the use of CLA in the diet. A significant statistical improvement was observed for the FC parameter ($P = 0.0160$) when the animals were fed a ration with 0.50% CLA as reported by (1) that observed an improvement in the feed efficiency of the 5.11% when 0.50% CLA was incorporated.

In the present study, improvement 4.09% in FC compared to control group implying significant feed savings at farm level. CLA inclusions in higher levels could generate greater productive benefits as mentioned by (1). According to (3) after the use of CLA, the reduction in FC may be due to the decrease in fatty tissue deposition through its ability to depress the activity of steroyl coenzyme A desaturase in porcine adipose tissue and adipocyte apoptosis.

Conclusions

The use of CLA in rations for finisher pigs generates productive benefits through reduction of FC. More studies are needed to determine the impact of CLA on tissues lipid profile and its subsequent impact on different aspects of human health as a nutritional alternative to generate functional foods.

Table 1

Parameters	Control	Test	P-value	VC%
IW, kg	92.12	92.21	-	-
FW, kg	129.70	130.91	0.3838	2.31
ADFI, kg	2.914	2.876	0.5414	5.31
ADG, kg	0.917	0.944	0.3876	7.90
FC	3.178	3.048	0.0160	4.17

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Different particle sizes in feed for finishing pigs with high slaughter weight

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Introduction

The pig industry is constantly evolving in search of increasing productive efficiency to improve competitiveness. With the reduction of corn particle size from 865 to 339 µm, an increase in energy digestibility was observed (1). As mentioned, milling processes improve the availability of nutrients for animals, resulting in an improvement in feed conversion and improvement in daily weight gain (1, 2). Other trials report that there was no improvement in the performance of the animals after a finer milling of the cereals, because there was a reduction in the daily feed consumption being that the author mentions that the palatability of the ground feeds below 600 µm is affected (3). In most of the published trials the animals were slaughtered between 100 and 115 kg of live weight, therefore the objective of this work was to evaluate the productive performance of pigs with a sales weight of 125 kg after the consumption of ground rations to 500 and 700 µm.

Material and methods

This trial was conducted at Biofarma Research Centre, Córdoba - Argentina. 120 pigs (castrated males and females - Naima x P76 Choice Genetics) were selected, distributed in random delineation with 2 treatments (T1 = 700 µm; T2 = 500 µm) with 6 pens per treatment and 10 animals per pen. The performance parameters evaluated were: Final weight (FW), average daily feed intake (ADFI), average daily gain (ADG) and feed conversion (FC). All parameters were subjected to an analysis of variance (ANOVA) through INFOSTAT® (National University of Córdoba, Argentina) with significant statistical difference P<0.05.

Results and Discussion

In this trial, a statistically significant difference was observed for ADFI (P = 0.0396) and FC (P = 0.0002). Animals fed with 500 µm ground feed had 5.66% lower ADFI and 5.02% better feed conversion than animals fed with 700 µm ground feed. For the rest of the parameters no significant statistical difference was observed (table 1). In the same way that (1,2) observed improvement in FC, this can be explained due to the increase in the surface area of smaller particles thus allowing greater possibility of action for digestive enzymes allowing greater availability of the main nutrients, including energy.

Conclusions

In conclusion, we can say that there is an improvement of 2.50% of FC for every 100 µm of particle size reduction. Therefore it is advisable to use feed with fine grinding in pigs in termination with high slaughter weight.

Table 1.

Parameters	700 µm	500 µm	*P-value*	VC%
IW, kg	31.98	32.00	-	-
FW, kg	129.30	128.55	0.7110	2.71
ADFI, kg	2.508	2.366	0.0396	4.20
ADG, kg	0.927	0.920	0.7149	3.64
FC	2.706	2.570	0.0002	1.49

Significant Statistical Difference P<0.05.

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Water consumption and its impact on suckling piglet performance

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Introduction

Water is an indispensable resource for all animal production. However, there are still some question when considering the piglets that have not yet been weaned.

The literature indicates that only breast milk would be enough as a complete diet for these animals (1, 2). However, it is not known whether dehydration caused by inadequate milk intake is a significant problem for piglets that are unable to develop normally, and even less information is available to understand if these animals would be able to recover their development when supplementary drinking water is provided.

Thus, this study was developed to evaluate the impact of water supply on the performance of suckling piglets.

Materials and Methods

Forty sows (Naima x P76 Choice Genetics) and their respective litters were selected and distributed in two treatments with 20 repetitions each. Piglets in the first group remained without access to water, while animals in the second group had free access to water during lactation phase.

Creep feeding was provided *ad libitum* to the piglets from the 10th day of life to the weaning. The water supply also started on the 10th day of life and was carried out using plate drinkers bolws fixed to the floor.

The performance of the litter was accessed by initial body weight, final body weight, total feed consumption, and pre-weaning mortality.

Variance test was used to compare the theatment, with a significance level of $P<0.05$.

Results

The performance of piglets with or without access to water is shown in Table 1. The performance of suckling piglets was not influenced by water supply ($P<0.05$).

Conclusions and Discussion

Pigs are curious animals that exhibit exploratory behavior from a very young age. When introduced to drinker devices, these animals may have very different reactions (fear, curiosity, play). After the initial phase, the presence of a new object (drinker devices) may had act as a environmental enrichment strategy. It is important to note that environmental enrichment can affect the behavior of piglets before weaning and also how they adapt after weaning (3). However, it was not observed in the present study.

The high milk production of the sows may also have influenced the water consumption of the piglets. Probably the milk produced by the sows in both groups was enough to supply the requirements of the litter during the study.

The facilities are another topic that may had influence on thewater intake by the piglets. The study was carried out in a facility with temperature control systems, so that there was no thermal discomfort for the animals. In general, this factor is a big problem faced by commercial farms, specially in tropical areas. Trus, it would be important to repeat the study in situations of thermal stress.

Previous studies have suggested that piglets with diarrhea may need more water than healtly ones (2), and it was also concluded that the water supply increased the survival of weak and underweight piglets as these animals would have difficulty competing for milk (4). However, this contition (health challange) was not tested in our study, which could help explaining the lack of significant results.

Table 1. Performance of suckling piglets with or without access to water.

Variable	No Water	With Water	*P-value*
Initial piglet number	13.300	13.250	0.9394
Initial litter weight, kg/litter	39.915	39.885	0.9921
Initial piglet weight, kg/piglet	3.050	3.049	0.9999
Final litter weight, kg	84.570	82.108	0.5750
Weaned piglet number	12.950	12.950	0.9999
Weaned piglet weight, kg	6.608	6.419	0.6045
Feed consumption, kg/litter	0.501	0.488	0.9052
Daily weight gain, kg/litter	3.435	3.248	0.2838

Considering the heterogeneity in the result of previous publications, further studies should be carried out in order to identify in which situations the consumption of water by the piglets is more important.

Acknowledgments

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Comparing manual and automatic feeding systems for lactating sows

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Introduction

The manual feeding systems are used worldwide for lactating sows. However, there are some issues related to the system, such as the amount of feed provided, the number of meals per day, the stress and increase in workforce needed. The use of automatic feeding equipment is increasing in pig farming worldwide, mainly focusing in pregnant sows. Several benefits are related to the adoption of precision feeding systems. The control of body weight is one of the most important advantage is sows (3), avoiding the presence of inappropriate body conditions and, thus, allowing the maintenance of adequate reproductive performance during the final stage of pregnancy, lactation and future cycles (1, 2). There is little scientific evidence on how the automatic feeding system could improve the performance of lactating sows in relation to manually fed animals. Therefore, this study was developed to evaluate the impact of automatic feeding on the productive performance of females and their respective litter during the maternity period compared to the conventional manual system.

Materials and Methods

Two hundred and eight sows and their respective litters (Naima x P76 Choice Genetics) were selected from parity order 1 to 7, and distributed in two treatments. The sows were housed in individual cages 5 days before farrowing. The animals in the first group (control) were fed manually, while those in the second group were fed using automatic feeding equipment (Nedap Compact Feeder, Netherlands). The water supply was *ad libitum*, and the amount of feed was offered according to the specifications for the animal's phase. Performance indicators were collected from both the sow and the litter. Variant test (ANOVA) was used to analyze the data. The parity order was also considered in the analysis.

Results

The productive performance of the sows and their litters are shown in Table 1. Feed lactating sows using an automatic equipment improved (P=0.0390) the weight gain of the litter and tended to increase the piglet at weaning (P=0.0790). The other responses were not influenced by the feeding systems.

Conclusions and Discussion

The feed supplied to the sows was similar in both treatments, however, animals fed manually probably showed higher waste of feed compared to the group automatically fed. Thus, the higher performance of the litter is due to the greater availability of milk, related to the greater feed intake. Another very important impact to be considered in the comparison is the optimization of workforce. Farmworkers spend much of their time feeding the animals. The automatic feeding systems are timesaving, which is important to focus in other segments of great importance, such as the birth monitoring and health checking. Technifying the labor is also a great opportunity when precisions feeding systems are adopted by the farm. So, respite most the performance results were not influenced by the system, it can be seen as an improvement in the productive efficiency of the farm. In this way, it can be concluded that the automatic feeding systems for lactating sows may be a good tool in the modern pig production. However, more studies are needed to improve feeding curves, meal frequency, among other feeding treats.

Table 1. Productive performance of lactating sows and their litters fed manually and automatically.

Response	Feeding system		*P-value*
	Manual	Automatic	
Replicate	98.000	110.000	-
Parity order, average	3.658	3.676	-
Total born, nº	15.510	15.427	0.6206
Born alive, nº	14.102	14.155	0.5715
Pre-weaning mortality, %	14.11%	13.33%	0.2068
Weaned piglets, nº	12.184	12.373	0.2660
Piglet weight at weaning, kg	7.620	7.722	0.0790
Daily litter weight gain, kg	3.829	3.942	0.0390
Sow weight loss, %	7.97%	9.76%	0.2175
Total feed supply, kg/sow	139.190	125.911	0.5051
Daily feed supply, kg/sow	6.223	5.660	0.9413

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Effect of an inactive high protein yeast (*Saccharomyces cerevisiae)* as a partial substitute of animal protein source in a prestarter diet for pigs

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Introduction

The newly weaned pig requires diets with highly digestible protein, which maximize the amount of amino acids reaching the bloodstream and muscle (1). Spray-dried animal plasma (SDAP) in addition to achieving this requirement, stimulates food consumption, modulates the immune system and improves intestinal structure (2); Nevertheless, despite its low inclusion (4 to 8%), the cost of the diet is significantly impacted (30 to 50%). Yeast *Saccharomyces cerevisiae* is a lower-cost protein source (≈ 46%), with an adequate amino acid profile (3). The objective of this experiment was to evaluate the dietary effect of an special inactive high protein content (50% mínimum) yeast *S. cerevisiae* as a protein source to partially replace the spray-dried animal plasma in the newly weaned pig.

Materials and methods

In this study, 192 weaned pigs (6.22 ± 0.5 kg) were used, housed in 6 pens (N = 32 pigs per pen / 3 pens per treatment). Piglets were randomly assigned to 2 different diets (treatments). The feeding period was as follows: Phase 1: d 1 to 13 ± 2; phase 2: d 14 to 20 ± 1; phase 3: d 21 to 41 ± 2. The control diet included 4, 2 and 1% spray-dried animal plasma (SDAP), for phases 1, 2 and 3, respectively. The experimental diet replaced 50% of SDAP in each phase, with inactive yeast (IY) *S. cerevisiae* (Nutrisaf ® 500, Phileo - Lesaffre), SDAP + IY: Phase 1: 2% SDAP + 2% IY; phase 2: 1% SDAP + 1% IY; phase 3: 0.5% SDAP + 0.5% IY. At the beginning and end of each phase, piglets were individually weighed and feed disappearance from the feeder in each pen was recorded. These data were used to calculate average daily weight gain (ADG), average daily feed intake (ADFI) and gain efficiency (G:F). Data were analyzed using a model for the analysis of variance for the design of repeated measurements, using the statistical package SAS 9.1, 2003 (SAS Inst. Inc., Cary, NC). Significance was set at $P < 0.05$. Trends were discussed at $P \geq 0.05$ to $P < 0.10$.

Results

Pigs fed with the SDAP diet had greater final body weight (P <.05) compared to the SDAP + IY group (23.46 vs. 22.68 kg, respectively). The ADFI of the pigs in the SDAP + IY diet was lower (P> .05) compared to the animals in the SDAP diet (0.55 vs. 0.62 kg / d, respectively). ADG was higher (P> .05) in piglets with SDAP treatment compared to animals in the SDAP + IY diet (0.42 vs. 0.40 kg / d, respectively). But interestingly G:F was improved (P <.05) in pigs with the SDAP + IY diet, compared to the SDAP diet (0.73 vs. 0.68, respectively). All the productive performance evaluated in the study is summarized in Table 1.

Table 1: Productive parameters (cumulative performance) d 1 - 41 by feed regimen.

Variable, kg	Treatments		SEM±	*P*-value
	SDAP	SDAP+IY		
BW d1	6.39	6.06	0.01	0.001
BW d 41	23.46	22.68	0.2	0.048
ADFI	0.62	0.55	0.02	0.058
ADG	0.42	0.40	0.01	0.440
G:F	0.68	0.73	0.03	0.021

SDAP = spray-dried animal plasma; IY = inactive yeast; SDAP + IY = spray-dried animal plasma + inactive yeast; BW= Body Weight.

Conclusion and discussion

There were no significant differences concerning to ADFI and ADG between the diet with spray-dried animal plasma and the partial replacement (50%) of SDAP by yeast protein (Nutrisaf ® 500, Phileo - Lesaffre); however, G:F was in favor of the animals fed by the diet with the inactive yeast, which implies that this protein source can be a viable replacement of SDAP in weaned piglet diets. Besides, by partially replacing SDAP, the cost of the diet decreased by 2.8%, and by 10.7% the cost per kg of weight gain.

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Effects of copper sulphate and dicopper oxide on growth performance and Cu liver accumulation when fed to growing pigs

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Introduction

Therapeutic doses of $CuSO_4$ (250 ppm Cu) improve growth performance and reduce the prevalence of diarrhea in pigs (1) but result in greater Cu accumulation in liver of weanling pigs compared with Cu_2O (2). There is no data about the effect of feeding therapeutic levels of Cu_2O on pigs' growth performance and Cu accumulation in liver during the growing and finishing periods. Therefore, the objective was to test the hypothesis that adding therapeutic levels of Cu as Cu_2O has the same effect on growth performance as $CuSO_4$, but results in less liver accumulation of Cu in growing and finishing pigs.

Materials and Methods

A total of 200 pigs (BW: 11.51 ± 0.98 kg) were allotted to 5 dietary treatments using a CRBD. There were 5 pigs per pen and 8 replicates per treatment. Treatments consisted of a negative control (NC) diet without Cu supplementation and 4 additional diets in which either 125 or 250 ppm of Cu from $CuSO_4$ or Cu_2O were added to the NC diet. Diets were based on corn and soybean meal containing 500 FTU/kg of phytase. The experiment was divided into 4 phases, with phase 1 lasting 28 d and the other phases lasting 35 d. Pigs were weighted individually at the start of the experiment and on the last d of each phase, feed intake was recorded in each phase. On the last d of phases 1 and 4, 1 pig per pen was sacrificed to obtain the liver for determination of Cu content.

Results

During the first 28 d, pigs fed a diet containing Cu had greater ($P < 0.05$) BW and ADG compared with pigs fed the NC diet. From d 28 onwards, pigs fed 250 ppm of Cu from Cu_2O had greater ($P < 0.05$) BW at the end of Phases 2, 3 and 4, had greater ($P < 0.05$) ADG in Phase 2 and greater ($P < 0.05$) ADFI in Phase 3. Overall, supplementation of 250 ppm of Cu_2O increased ($P < 0.05$) BW, ADG and ADFI (Table 1).

Table 1. Growth performance of pigs fed the dietary treatments for the global experiment (from 11 to 120 kg BW).

	NC	$CuSO_4$		Cu_2O		SEM
		125	250	125	250	
BW	117^{b}	117^{b}	116^{b}	117^{b}	122^{a}	1.9
ADG	0.89^{b}	0.91^{ab}	0.89^{b}	0.91^{ab}	0.95^{a}	0.02
ADFI	2.18^{ab}	2.15^{b}	2.12^{b}	2.17^{b}	2.33^{a}	0.05
G:F	0.42	0.42	0.42	0.42	0.41	0.01

[a,b] values within a raw without a common superscript are different ($P < 0.05$).

There were no differences in liver weight among treatments. At the end of Phase 1, pigs fed 250 ppm of Cu from $CuSO_4$ had the greatest ($P < 0.05$) Cu concentration in liver compared to the others treatments (Figure 1). At the end of Phase 4, pigs fed 250 ppm of Cu had greater ($P < 0.05$) Cu concentration in liver compared with pigs fed 125 ppm of Cu or the NC diet.

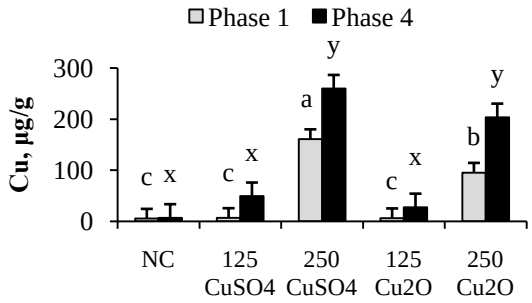

Figure 1. Liver copper concentration (µg/g) in pigs fed dietary treatments.
[a,b,c] values in Phase 1 without a common superscript are different ($P < 0.05$).
[x,y] values in Phase 4 without a common superscript are different ($P < 0.05$).

Discussion and Conclusions

Inclusion of both sources of Cu improved growth performance of pigs during Phase 1, which is in agreement with (2), but only pigs fed 250 ppm Cu from Cu_2O had better growth performance in growing and finishing phases, which may be related to modification of the intestinal microbiota (3, 4, 5). Less Cu concentration in the liver of pigs fed the diets containing Cu_2O may be a result of reduced Cu absorption from the oxide form or a greater Cu excretion in bile or urine. Further analyses are in progress to elucidate the fate of Cu in the body.
To conclude, growing pigs fed diets supplemented with 250 ppm of Cu from Cu_2O perform better compared with pigs fed $CuSO_4$ and with less Cu accumulation in the liver.

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Effect of Oregano Essential Oil on Progeny Health and Performance of Supplemented Sows

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Introduction

There is increasing pressure on the pig industry to reduce the use of antimicrobials whilst improving animal performance and welfare, driving recent interest in sustainable alternatives. Oregano essential oil (*Origanum vulgare*) contains the active compounds carvacrol, thymol, y-terpinene and p-cymene, which possess both anti-microbial and anti-oxidant activity (Hammer et al., 1999). In a previous study, oregano essential oil was shown to be able to support sow reproductive performance and help wean a greater number of more robust piglets (Tan et al., 2015).

Materials and Methods

Over the course of two batches, two hundred LW x LR sows were randomly allocated to either control (CON) or oregano essential oil supplementation (OS) and balanced for parity at service. Treatments either received CON, basal gestation and lactation diets, formulated to meet or exceed NRC requirements (NRC, 2012) or OS, basal diets supplemented with 500g/t oregano essential oil (Orego-Stim, Anpario Plc., UK) from service, throughout gestation and lactation to weaning (~19 days). All piglets were ear-tagged at birth and cross-fostered to fill sows to teat count. Born alive, dead, mummified and stillborn were recorded. Litter weight was measured at birth, day two and day nineteen to evaluate numbers born, weaned and litter growth for each treatment. Milk samples were collected from 30 sows (15 per treatment) within 48 hours of farrowing and day 10 of lactation for IgA and IgG analysis. Sow, litter, and individual performance was analyzed as a completely randomized design using PROC Mixed procedure and mortality, falloffs, and piglets removed were analyzed with PROC Glimmix 3 procedure of SAS 9.4 (SAS, Cary, NC). Number of piglets weaned analysed per treatment by relative risk assessment, GLM.

Results

Average number of piglets born alive was conserved across both treatment groups (14.61 vs 14.36 for CON and OS respectively). At weaning, average piglet weight was similar but litter weight was numerically heavier for OS supplemented sows due to an increased number of piglets weaned. Removals (mortality and culls) showed a trend for reduction (p= 0.05) following OS supplementation and a 2% reduction in pre-weaning mortality compared to the control (11.13 vs 9.09 for CON and OS respectively) (Fig. 1). This resulted in a significantly increased number of weaned piglets (by 11%) Fig 2. As analysed by relative risk of survival to weaning (rr=1.73 (p=0.0001).

Conclusions and Discussion

These differences provide an economical benefit for the unit with an increased number of piglets weaned per sow, providing a potential margin over feed benefit of £59 per sow per year.

Eubiotics such as Orego-Stim could provide a natural tool for the improvement of sow and progeny health and performance which may have a significant effect on lifetime performance and medication use.

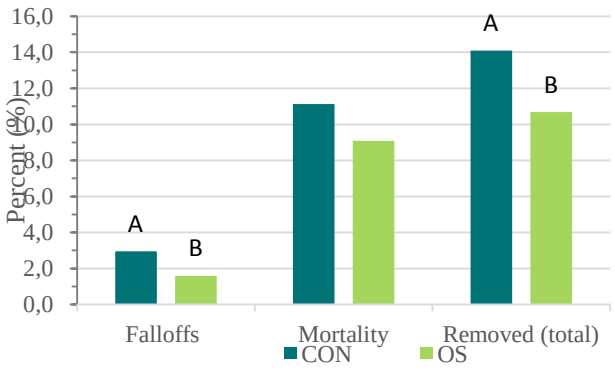

Figure. 1. Piglet losses and removals per treatment (A-B letters denote statistical trend of significant difference by p<0.1)

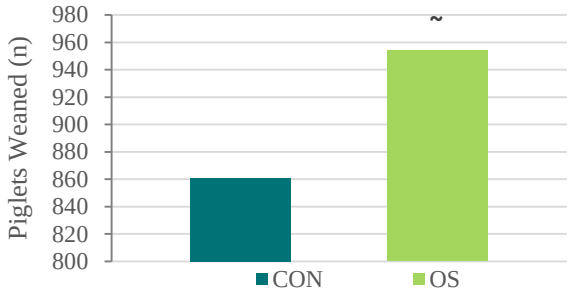

Figure 2. Piglets weaned per treatment (~represents significantly improved RR=1.73, p=0.0001))

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Transcriptional response of serotonin receptors to weaning and feed additive intervention in pigs

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Introduction

Feed additives such as fermented cereals, some edible mushrooms, and organic acids supplemented to piglet diets support intestinal health post weaning by maintaining epithelial integrity, beneficial bacterial communities, and immune system function.

Serotonin (5-HT), is a recognized neurotransmitter with key roles in the gastrointestinal tract. When activated, the family of 5-HT receptors (HTR3, 4 and 7) have be shown to increase motility, mucus discharge, and chloride secretion. Functions appear generally pro-inflammatory although signaling can also support anti-inflammatory pathways (1). Expression of 5-HT receptors can help understand intestinal homeostasis and health, however, information in piglets is limited.

The present objectives were to A) describe intestinal HTR gene expression in nursery pigs, and B) evaluate two dietary treatments as 1) FS, a fungal (*Agaricus subrufescens)* fermented rye plus hydrolyzed copra meal, and 2) FA, a feed additive blend (with organic acid including encapsulated butyrate) for effectiveness on the mitigation of post-weaning intestinal disruption via gene expression.

Materials and Methods

The experiment was conducted in University of Murcia (Spain) research facilities. Post-weaning, 100 nursery pigs were randomly assigned to control (n = 40), FS (2 kg/MT; n = 30), or FA (2 kg/MT; n = 30). Treatments were dietary and feed was provided ad libitum for 45 days. Intestinal tissue samples (jejunum and colon) were collected from 10 control pigs after weaning (day 1) and 10 pigs per treatment at day 15 and 45 and used to determine HTR3, HTR4 and HTR7 gene expression. The sequences used were XM_003357301.4, NM_001001267.1 and NM_214085.1 for receptors 3, 4 and 7, respectively. Primers pairs were designed using Primer Blast® (National Center for Biotechnology Information, MD, USA). The PCR quantitation was corrected by means of Pfaffal method, using the efficiency of the PCRs. The normalized expression of the HTR genes was compared to 1) day 1 or 2) the control treatment at each time point. Statistical analyses were conducted using Kruskal-Wallis test using SPSS v. 23 software. Pearson correlation was used to evaluate the relationship between HTR receptor expression from colon and jejunum tissue samples.

Results

In the jejunum of pigs 1 day post-weaning, HTR3 was highly upregulated relative to day 15 and 45 for all dietary treatments ($P < 0.001$). Similarly, HTR4 was upregulated on day 1 compared to day 15 for the control and FS treatments, and higher than control and FA treatments at day 45. Expression of HTR7 at day 1 was upregulated compared to day 15 and day 45 ($P < 0.02$), with the exception of the FA treatment showing greater upregulation at day 45 ($P<0.002$).

In colon, fewer temporal and treatment differences were found. Expression of HTR4 at day 45 was lower than control at day 1 in response to the FA treatment ($P = 0.025$). Relative to day 1, expression of HTR7 at day 15 was upregulated in the FA group ($P < 0.001$), while on day 15, HTR7 was down regulated for FS compared to control ($P = 0.043$).

Comparisons across tissue type and over time revealed few differences among dietary treatments. In colon tissue at day 45, HTR4 showed a tendency ($P = 0.089$) to be down regulated in the FA group compared to control. At day 15, HTR7 expression tended to be lower in response to the FS treatment relative to control in the jejunum ($P = 0.073$) and colon ($P = 0.075$), while expression was upregulated in colon tissue in the FS group compared to control and FA treatments ($P = 0.048$).

A significant but weak correlation was found between HTR3-HTR4-HTR7 expression profiles in jejunum ($0.38 < r < 0.50$; $P < 0.01$) but not in colon.

Conclusions and Discussion

Following weaning, the expression of serotonin receptor genes was increased at day 1 compared with that on days 15 and 45 in jejunum tissue. As per previous findings with similar results, it was suggested that there is an increased sensitivity and capacity for serotonin signaling post weaning, as indicated by increased receptor expression (2). The temporal expression profile of HTR genes in the colon suggest a more limited role of serotonin signaling post weaning.

Transcriptional responses to FS and FA treatments are indicative of the potential to modulate serotonin receptor gene expression post weaning. Further research on HTR expression and the relationship with gut physiology, health and immunity is warranted to better understand the role of these receptors in the gastrointestinal tract of pigs and the potential for nutritional intervention.

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Diarrhea incidence and fermentative end-products in feces of pigs fed diets containing rapidly or slowly digestible protein sources and fermentable or resistant dietary fiber sources

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Introduction

Protein fermentation by intestinal bacteria in nursery pigs appears to be a major cause of post-weaning diarrhea. Providing weanling pigs with diets containing 14 to 17% crude protein has been reported to reduce post-weaning diarrhea compared with diets containing 19 to 22% crude protein (1). Furthermore, feeding diets containing different sources and quantities of dietary fiber have also reduced post-weaning diarrhea (2). Therefore, it was hypothesized that both protein and dietary fiber sources could interact and be used to reduce post-weaning diarrhea.

Materials and Methods

A total of 240 weaned piglets (24 days of age) were randomly allotted in a completely randomized design to 4 dietary treatments according to body weight (BW) and sow parity. Treatments were arranged in a 2 × 2 factorial arrangement with 2 speeds of *in vitro* protein digestion kinetics (rapid or slow) and 2 types of dietary fiber (fermentable or resistant). There were 6 pigs per pen and 10 replicate pens per treatment. The male:gilt ratio was equal among pens within a replicate. There were 3 experimental phases; 1 from d 0 – 10, 2 from d 10 – 28, and 3 from d 28 – 42 post-weaning, respectively. Feed and water were provided *ad libitum*. Pig BW, ADG, ADFI, and G:F were calculated for each phase. Feces scores were recorded for each pen daily.

On d 9 and 41 post-weaning, a fecal sample was collected from 1 pig per pen (40 pigs total) via rectal stimulation. The pH of each sample was immediately measured and a sample was analyzed for volatile fatty acid concentrations using gas chromatography and total eubacteria using qPCR 16s rRNA sequencing.

The treatment contrasts were similar in each experimental phase. All diets were formulated to be isocaloric and isonitrogenous and were formulated with commercial feed ingredients varying in relative *in vitro* rates of rapidly/slowly digestible protein and fermentable fiber levels to meet or exceed requirements for standardized ileal digestible (SID) AA, standardized total tract digestible (STTD) P, vitamins, and minerals (3). An attempt was made to ensure the concentrations of total dietary fiber and phytate in the diets were similar. No exogenous enzymes were added to the diets.

Results and Discussion

The ADFI of nursery pigs fed diets containing resistant fiber was 593 g/d and this was less ($P < 0.05$) than pigs fed diets containing fermentable fiber (628 g/d). This resulted in a reduced ($P < 0.05$) ADG (456 g/d) in pigs fed resistant fiber diets compared with pigs fed fermentable fiber diets (489 g/d). Diarrhea incidence in each phase, and overall, was less ($P < 0.05$) when pigs were fed resistant fiber diets compared with fermentable fiber diets. During phases 2, 3, and overall, pigs fed diets containing slowly digestible protein sources had a greater ($P < 0.05$) incidence of diarrhea compared with pigs fed diets containing rapidly digestible protein sources. Pigs fed diets containing slowly digestible protein sources tended to have a greater ($P < 0.10$) fecal pH on d 9 post-wean compared with pigs fed diets containing rapidly digestible protein sources. Protein fermentation results in the formation of branched-chain fatty acids, phenols, indoles, skatoles, biogenic amines, ammonia, among other metabolites and the majority of these are basic and tend to increase pH. Indeed, protein fermentation was greater in pigs fed diets containing slowly digestible protein sources as indicated by a greater ($P < 0.05$) concentration of iso-butyric and iso-valeric acid in feces on d 41 compared with pigs fed diets containing rapidly digestible protein sources. Pigs fed diets containing slowly digestible protein sources, or resistant fiber sources, had greater ($P < 0.05$) total bacteria on d 41 in feces compared with pigs fed diets containing rapidly digestible protein sources or fermentable dietary fiber sources. More bacteria in feces is indicative of greater substrate availability. It is preferred that microbes ferment carbohydrates rather than protein, thus resistant fiber sources ensure carbohydrates are not exhausted by the end of the large intestine.

Conclusions

Diarrhea incidence was reduced in pigs fed diets containing *in vitro* rapidly digestible protein sources or resistant fiber sources. Protein fermentation in the large intestine was greater in pigs fed diets containing *in vitro* slowly digestible protein sources. Therefore, it is recommended that nursery pig diets contain *in vitro* rapidly digestible protein sources and resistant fiber sources to reduce post-weaning diarrhea.

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Effect of two yeast strains inclusion on the productive performance of piglets challenged with *E. coli* K88$^+$ in the nursery phase

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Introduction

Post-weaning diarrhea, associated with the colonization of enterotoxigenic *E. coli* (1), causes poor growth performance in piglet. Thus, the use of live yeasts as probiotics (*Saccharomyces spp.*) has been an alternative in swine production (2). It is a technology that promotes efficiency in production parameters (3,4) and improves health (5). However, results vary between experiments and some have not shown changes associated with the use of probiotics. The objective of the present trial was to evaluate the effect of two live yeast probiotics (LYP) (*Saccharomyces cerevisae* yeast strains) diet inclusion on the performance of nursery piglets challenged with *E. coli K88*$^+$ (6).

Materials and Methods

One hundred and ninety-two piglets weaned at 23 days of age were divided into four experimental groups using a randomized block design (initial weight and sex), totaling 12 replicates with four pigs per experimental unit. The experimental treatments were: T1: no LYP and not challenged; T2: LYP strain 1 and challenged; T3: LYP strain 2 and challenged; T4: with no LYP and challenged. LYP were included in the diet to replace kaolin throughout the experiment. On days eighth, ninth of the trial the challenged piglets received 1 ml of inoculum at a concentration of 10^6 CFU per ml with *E. coli K88*$^+$ and on day seventeenth challenged pigs received 2ml of inoculum at a concentration of 10^9 CFU. Piglets in the non-challenged group received the same amount of saline solution on the same days of the challenged. The experiment lasted 42 days and the piglets were weighed weekly. The variables daily weight gain (ADG), average daily feed intake (ADFI) and gain-to-feed ratio (G:F) were evaluated in the periods from 0-7, 8-28 and 29-42 days. LSMEANS were compared by Tukey test with P<0.05 by SAS MIXED procedure.

Results

The results are shown in Table 1. Piglets from T2 presented a 7% heavier body weight at 7d (P=0.031), 15.2% greater ADG 0-7d (P=0.033), and 9.5% better G:F 0-7d compared with piglets of T4 group. Similarly, for the 8-28d period, the piglets from T2 group presented a greater live weight at 28d of 7.7% (P <0.01), greater ADG 8-28 days of 11.3% (P<0.01), and greater ADFI of 12.3% (P=0.012) compared to piglets from T4 group. From days 29-42 no significant treatment effects were observed (P > 0.10).

Table 1. Performance of nursery piglets challenged with *E. Coli* K88$^+$ receiving yeast supplemented diets

Variables (kg)	Treatment[1]				P value
	1	2	3	4	
Initial weight	6.71	6.70	6.70	6.70	0.625
Weight 7 days	8.70ab	8.83a	8.75ab	8.54b	0.031
ADG, 0-7 days	0.284ab	0.304a	0.293ab	0.264b	0.033
ADFI, 0-7 days	0.383	0.400	0.391	0.385	0.605
G:F 0-7 days	0.762ab	0.752a	0.742a	0.687b	0.009
Weight 28 days	18.61ab	19.26a	18.66ab	17.88b	0.001
ADG, 8-28 days	0.470ab	0.493a	0.472ab	0.443b	0.002
ADFI, 8-28 days	0.715ab	0.756a	0.719ab	0.673b	0.012
G:F 8-28 days	0.661	0.657	0.663	0.659	0.975
Weight 42 days	26.01	26.48	26.28	25.72	0.322
ADG,29-42 days	0.528	0.504	0.520	0.534	0.680
ADFI,29-42 days	0.937	0.958	0.960	0.971	0.900
G:F 29-42 days	0.573	0.527	0.546	0.554	0.382

[1]ADG: average daily gain; ADFI: average daily feed intake; G:F: gain-to-feed ratio; Line averages followed by different letters differ by Tukey's test with P <0.05.

Conclusions and Discussion

LYP strain SC47 from Phileo by Lassafre diet inclusion proved to be effective when compared to the other treatments, especially in the first three weeks, considered the most challenging of the productive system. Thereby, LYP *Saccharomyces cerevisae* can minimize the deleterious effects associated with *E. coli* K88$^+$ challenge in weaned piglets.

Acknowledgments

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Yeasts strains fed as probiotic do not alter the blood cell composition on post weaning piglets challenged with *E. coli* K88$^+$

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Introduction

After the World Health Organization prohibited the use of antibiotics as growth promoters (AGP), the need for viable alternatives arose. Probiotics, among them live yeasts, have been subject to several recent studies[1–3] and shown great potential as substitutes of AGP. The use of yeasts has been associated with increased ADG, mucosal macrophage count and gut health[1–3] although few studies have analyzed its effects on blood cell composition (BCC)[3]. This study aimed to evaluate the effect of two strains of yeasts on the blood cell composition of piglets challenged with *Escherichia coli* strain K88$^+$

Materials and Methods

One hundred and eight piglets were separated in 4 experimental treatments: T1: no yeast strain and not challenged; T2: yeast strain 1 and challenged; T3: yeast strain 2 and challenged; T4: with no yeast strain and challenged.

The 42 days of experiment were divided based on the diet fed to the animals: days 0 to 7 (pre-starter 1), 8 to 28 (pre-starter 2) and 29 through 42 (starter). During the pre-starter 1 period 200 mg/kg of doxycycline was included and used as a preventive antibiotic. Additionally, zinc oxide was provided during the first week (3000 mg/kg) and the pre-starter 2 period (2300 mg/kg). Initially 2,0 kg/ton of the yeast strains were included in the diet, decreasing to 1 kg/ton on the pre-starter 2 and starter period, always replacing kaolin in the formulation.

On the eighth and ninth days, piglets from T2, T3 and T4 received 1 ml of solution containing 10^6 CFU / ml of *Escherichia coli* and on the 17th day 2 ml of solution containing 10^9 CFU / ml of *E. coli*; at the same dates, pigs from the group T1 were inoculated with identical volumes of saline solution.

Blood was collected during the slaughters, at the 11th, 28th, and 42nd days. In each day 36 animals, 9 animals per treatment were euthanized, totaling 108 animals. BCC was made in the Laboratory on the Hospital Clinical Teaching unit of the University of São Paulo and ANOVA was evaluated using SAS PROC MIXED. Values were considered different when P value was less than 0.05.

Results

The parameters measured on the blood cell composition evaluation were total red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, neutrophils, lymphocytes, monocytes, eosinophils, basophils, platelets, plasmatic protein and neutrophil/lymphocyte ratio.

There were no statistical differences between treatment groups for all evaluated parameters ($P > 0.05$). All blood cell composition parameters were between the expected range for the species.

Conclusions and Discussion

Other studies[3] also didn't find any influence of dietary treatment on BCC.

The neutrophil/lymphocyte ratio was used as an stress indicator as suggested by other studies[5,6]. The values found were similar to those described by Widowski[5] in animals with higher blood concentration of cortisol. The subjects were exposed to possible stress factors (transportation, weighting procedures) immediately before the slaughter, which might be a possible reason for these greater values.

The results found suggest that feeding yeast strains for weaned piglets has no effect on the blood cell composition.

Acknowledgments

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Silver nanoparticle as a growth promoter for finishing pigs improves carcass yield

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Introduction

The use of silver nanoparticles can be an interesting technology to replace antibiotics administered as growth promoters, since even in low concentrations it has a broad spectrum of antibacterial action, improving the intestinal health of animals. The objective of this study was to evaluate the possible use of silver nanoparticles to replace pig growth promoters in the growing and finishing phases.

Materials and Methods

The study was conducted at the Swine Research Laboratory (LPS) of FMVZ/USP - *Campus* Pirassununga/SP. Eighty pigs (barrows and gilts) at 70 days of age with an average live weight of 24.09 kg were used in the trial. Pigs were housed in pens in the growing/finishing unit. The experimental design was a randomized block (initial weight and sex) with five treatments and eight replicates. The experimental unit was composed by the pen (average of two animals). The experimental period was 83 days divided into two periods according to feed changes: growing (0 to 42 days) and finishing (43 to 83 days). Treatments used were: NC: Negative control without growth-promoting additive; PC: Positive control with Halquinol as a growth promoter at a dose of 120 ppm; NP5: Use of silver nanoparticle as a growth promoter at a dose of 5 ppm; NP10: Use of silver nanoparticle as a growth promoter at a dose of 10 ppm; NP15: Use of silver nanoparticle as a growth promoter at a dose of 15 ppm. The animals were weighed at the beginning of the experiment, at 42nd and 83rd days of experiment. The feed provided and leftovers were evaluated daily. Based on these data, average daily gain (ADG), average daily intake (ADI) and feed conversion (FC) were calculated. At the end of the experiment, all animals were sent to slaughter for carcass evaluation. The data were submitted to the statistical package of the SAS software (2009) through the MIXED procedure.

Results

The results are shown in Table 1, no effect were observed, just a trend was observed for carcass yield, in which animals that received silver nanoparticles at a dosage of 5 ppm have a greater yield.

Table 1. Performance of swine receiving diets containing silver nanoparticles

Variables	CN	NP5	NP10	NP15	CP	CV, %	P value
Initial weight, kg	24.08	24.09	24.10	24.08	24.09	10.86	0.999
Weight 42 days, kg	61.38	62.64	58.47	61.02	62.03	9.94	0.125
ADG 0-42 days, kg	0.889	0.919	0.820	0.879	0.903	11.38	0.148
ADFI 0-42 days, kg	2.180	2.153	2.048	2.269	2.260	12.70	0.337
FC 0-42 days, kg	2.466	2.350	2.504	2.575	2.505	8.03	0.350
Weight 83 days, kg	97.44	97.01	94.96	97.11	96.74	6.99	0.689
ADG 43-83 days, kg	0.880	0.836	0.890	0.879	0.848	8.96	0.496
ADFI 43-83 days, kg	2.940	2.719	2.754	2.923	2.714	13.90	0.258
FC 43-83 days, kg	3.344	3.248	3.091	3.333	3.208	11.54	0.533
ADG 0-83 days, kg	0.884	0.880	0.854	0.880	0.875	7.30	0.704
ADI 0-83 days, kg	2.558	2.433	2.395	2.593	2.485	11.19	0.163
FC 0-83 days, kg	2.891	2.765	2.803	2.949	2.833	7.41	0.340
Carcass weight, kg	66.89	67.13	65.60	66.38	64.70	6.98	0.599
Carcass yield, %	68.06	69.69	68.21	68.45	67.40	2.29	0.059

NC: Negative control; NP5: Inclusion of 5 ppm of silver nanoparticle; NP10: Inclusion of 10 ppm of silver nanoparticle; NP15: Inclusion of 15 ppm silver nanoparticle and PC: Positive control with Halquinol as a growth promoter at a dose of 120 ppm; ADG: Average daily gain; ADFI: Average daily feed intake; FC: Feed conversion.

Conclusions and Discussion

The beneficial effects of including silver nanoparticles in the growing / finishing pig diet were verified with the inclusion of 5 ppm of the product which is associated with the nanoparticle ability to improve digestibility, increase intestinal development, increase mineral absorption and its antimicrobial properties (1), leading to better use of the offered diet due to its potential to increase efficiency in the digestion and absorption process, in addition to the control of pathogenic bacteria. The use of silver nanoparticles is considered a promising technology; however, further studies are needed to improve its use.

Acknowledgments

USP Swine Research Lab and Brazilian Nano Feed

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Silver nanoparticle improves the fecal score of pigs in the growing and finishing phase

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Introduction

The excessive use of antimicrobial agents promotes the emergence of resistant microorganisms, which are harmful to animal and human health. The use of silver nanoparticles can be a possible substitute for antibiotics administered as growth promoters, as it has a broad spectrum of antibacterial action, improving the intestinal health of animals. The objective of this study was to evaluate the use of silver nanoparticles in promoting health through score fecal evaluation in replacement of antibiotic growth promoters during growing and finishing phases of pigs.

Materials and Methods

The study was conducted at the Swine Research Laboratory (LPS) of FMVZ/USP - *Campus* Pirassununga/SP. Eighty pigs (barrows and gilts) at 70 days of age with an average live weight of 24.09 kg were used in the trial. Pigs were housed in pens in the growing/finishing unit. The experimental design was a randomized block (initial weight and sex) with five treatments and eight replicates. The experimental unit was composed by the pen (average of two animals). The experimental period was 83 days divided into two periods according to feed changes: growing (0 to 42 days) and finishing (43 to 83 days). Treatments used were: NC: Negative control without growth-promoting additive; PC: Positive control with Halquinol as a growth promoter at a dose of 120 ppm; NP5: Use of silver nanoparticle as a growth promoter at a dose of 5 ppm; NP10: Use of silver nanoparticle as a growth promoter at a dose of 10 ppm; NP15: Use of silver nanoparticle as a growth promoter at a dose of 15 ppm. Once a day, throughout the experimental period, the analysis of the fecal score was performed through the classification of feces in the pen and the occurrence of diarrhea was calculated as a percentage related to the study period. Diarrhea was verified by presence of liquid or pasty stools, as proposed by (1). The diarrhea incidence variable was analyzed using the binomial generalized linear model in the SAS GENMOD procedure.

Results

The results are shown in Table 1. The lower incidence ($P<0.05$) of diarrhea (score 3) was observed among the animals that received the positive control (PC). The animals receiving silver nanoparticles presented an intermediate value in relation to the diarrhea index.

Table 1. Fecal score of pigs receiving diets containing silver nanoparticles during 12 weeks

Variables	Treatments					CV, %	P value
	NC	NP5	NP10	NP15	PC		
Score 1, %	11.48	14.42	14.25	18.76	14.08	67.36	0.173
Score 2, %	40.93^{b}	49.18^{ab}	53.75^{ab}	52.99^{ab}	60.59^{a}	23.81	0.034
Score 3, %	41.06^{a}	32.32^{ab}	27.75^{ab}	24.85^{ab}	22.87^{b}	51.41	0.097
Score 4, %	6.53	4.07	4.25	3.40	2.46	95.59	0.428
Fecal score	2.43	2.26	2.22	2.13	2.14	13.21	0.128

Fecal score: Weighted average of fecal scores; CV: coefficient of variation; Line averages followed by distinct lower-case superscript letters differ by Dunn's test with $P<0.10$.

Conclusion and Discussion

Beneficial effects of the silver nanoparticles and halquinol were observed, improving the fecal score. However, halquinol was the additive that showed the best efficacy, this can be explained by the fact that it has a greater affinity for lipids making it easier for the molecules to penetrate the microbial membrane (2), exercising the antimicrobial role and acting on gram positive and negative bacteria (3) inhibiting their growth and proliferation. The nanoparticle has a bacteriostatic and bactericidal action, thus minimizing the challenge caused by pathogenic microorganisms, which demonstrates its ability to minimize the incidence of diarrhea. The silver nanoparticle is a promising growth promoter, however, more studies are needed to improve the use of this technology.

Acknowledgments

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Does glycemic concentration of the parturient sow affect farrowing kinetics?

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Introduction

Years of selection of breeding sows for prolificacy resulted in larger litter size, and high rate of pre-weaning mortality (1). Larger litter size is associated with prolonged farrowing duration, which in turn increases the incidence of asphyxiated piglets and the stillbirth rate. Moreover, prolonged duration of farrowing compromise sows' health and fertility (2). During farrowing, the gravid uterus is reliant on energy from glucose oxidation to support its intense contractions. Therefore, sows with adequate blood glucose concentration at the onset of farrowing are more likely to have improved farrowing processes. Thus, we investigated whether blood glucose concentration would affect farrowing kinetics.

Materials and Methods

80 hybrid parturient sows, with parity ranging between 0 and 8 had their farrowing fully monitored. The farrowing duration (FD) was defined as the time elapsed between the birth of the first-born and the last-born piglet in the litter. Birth Interval (BI) was obtained by dividing the FD by the number of piglets born in the litter. Plasma glucose concentration was assessed using a glucometer (Accu-Chek Guide Meter™, Roche Diabetes Care, Inc). Blood samples were collected by puncture of the auricular vein. Plasma glucose was measured in two moments: initial glycemia (birth of the first piglet), and final glycemia (beginning of expulsion of the placenta). Mean glycemic concentration was calculated as the arithmetic average of the initial and final glycemia. Sows were divided according to their glycemic concentration in three groups: Low (≤3.89 mMol/L), n = 15; Intermediate (3.90 - 4.99 mMol/L), n = 46; and High (≥5.00 mMol/L), n = 19. All variables were tested for normality. Pearson's correlation test was used to analyze correlations between FD and mean glycemic concentration. Mean FD and BI were analyzed by Tukey's test (SAS® Enterprise Guide 4.3, Cary, NC, USA).

Results

FD and mean glycemic concentration are negatively correlated (P<0.001 and r = -0.39 for Pearson correlation (Figure 1)). Mean glycemic concentration had a statistically significant effect on the FD and BI as shown in table 1.

Discussion and Conclusion

Farrowing duration exceeding 300 min is considered prolonged, impairing sow's and piglet's health. Indeed, sows with prolonged farrowing duration had a 275% increase in stillbirth rate (3). In this study, low glycemic sows had a mean farrowing duration of 359 minutes. This demonstrates that glycemic concentration is a key factor affecting farrowing kinetics traits. Therefore, strategies to maintain a proper glycemic concentration during farrowing warrants new research.

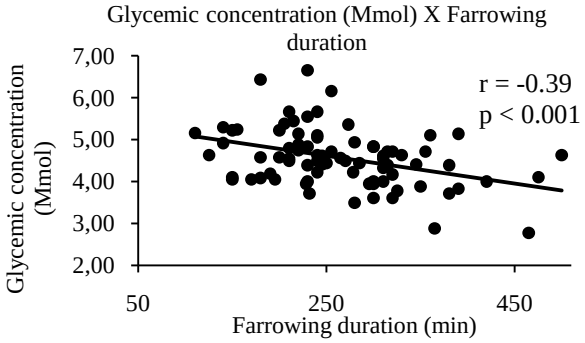

Figure 1. Pearson correlation between glycemic concentration and farrowing duration

Table 1. Mean FD and BI according to low, medium, and high glycemic concentration of the parturient sow

	Glycemic concentration			
	Low ≤3.89	Intermediate 3.90 - 4.99	High ≥5.00	P
N	15	46	19	-
FD (min)	359[a]	262[b]	226[b]	0.001
BI (min)	20.6[a]	16.8[b]	14.6[b]	0.070

FD: Farrowing Duration; BI: Birth Interval
(a, b) Superscripts indicate statistically significant differences by Tukey test with P<0.05

Acknowledgments

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Weight and litter uniformity improvement in sows that received butaphosphan + vitamin B12 injectable supplement

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Introduction

Hyperprolific sows are interesting in pig production. However, a higher number of piglets per litter usually means low individual piglet birth weight in litters with high variation in birth weights (1, 2). Solutions that improve litter uniformity are desirable, as there are pre and post-weaning mortality reduction as well as improved growth performance after weaning (3). The aim of this study was to test the influence of a butaphosphan + vitamin B12 injected supplement in sows on litter uniformity.

Materials and Methods

22 parity 1 to 3 sows received tree doses of butaphosphan 100mg + vitamin B12 0,05mg/ml (the first dose at the day sows were allocated in the farrowing barn, ~ 112th day of pregnancy; the second dose when the first piglet was born; third dose at weaning – piglets were 21st day of life. Each time sow received 10ml intramuscular injection. A control group (n=21) had phosphate buffer saline injections. Piglets were individually weighted in the 1st and 21st days of life.

To analyze data, we created categories of piglet's weights (categories for weights at 1st day of life: <1kg,≥1 to <1.5, ≥1.5 to <2kg and >2kg; categories for 21th days of life: <3kg, ≥3 to <4.5kg, ≥4.5 to <6kg and ≥6kg). We used the Qui-square test (significance level = 0.05) and calculated adjusted residuals (AR). If AR was greater/ less than +/- 1.96, the observed frequency was greater/less than the expected frequency (4).

Results and discussion

Piglets from supplemented sows had greater weight uniformity compared with piglets from the control group sows. Results are shown in figures 1 and 2. Piglets from supplemented sows: observed frequencies were greater than the expected frequencies in categories ≥1kg to <1.5kg (1st day of life) and ≥4.5 to <6kg (21st day of life), both with more than half of the piglets analyzed.

Piglets from the control group: observed frequencies were greater than the expected frequencies in categories <1kg, >2kg (1st day of life), <3 and ≥3kg to <4.5kg (21st day of life), but all with less than 29.9% of the piglets analyzed.

We can also observe a displacement of the orange columns to the right in both Figures 1 and 2, showing piglets from supplemented sows also weigh more than the control group.

There are few studies on injectable supplementation in sows and improvement of litter uniformity. Some studies added supplements to the sows' diet, such as organic minerals (5) and arginine (6), and observed better litter uniformity at birth and weaning.

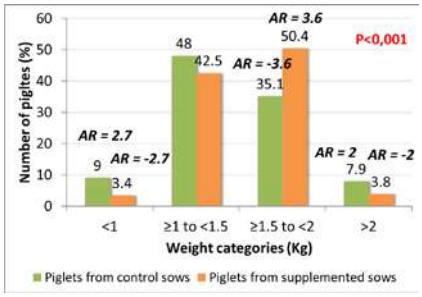

Figure 1. Distribution of piglets per weight in categories - 1st day of life.

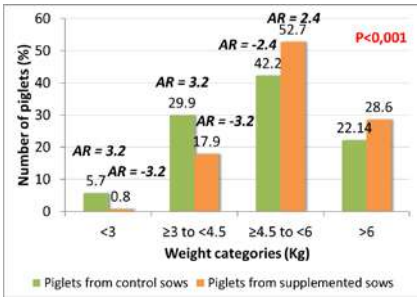

Figure 2. Distribution of piglets per weight in categories – 21st day of life.

Sows with good nutrient attendance produce higher quality and higher amounts of colostrum and milk for newborns (3, 7). Therefore, we can assume that butaphosphan + vitamin B12 injection increased the availability of phosphorus and B12, which probably enable better destination of these nutrients for colostrum and milk.

Conclusions

Injectable butaphosphan + vitamin B12 improved weight and litter uniformity.

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Effect of increasing feed intake at late gestation on the sow performance

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Introduction

The increase of prolificacy is associated to lower mean and higher variability of piglet's birth body weight (BBW) together with higher pre-weaning mortality (4). Increasing sow feed allowance in later gestation may be a strategy to increase the mean BBW (1). However, other authors (2,3), reported a higher farrowing length and a lower feed intake during lactation after increasing feed intake in late gestation. Therefore, the objective was to study the effect of increasing feed intake the last three weeks of gestation on farrowing and lactation performance.

Materials and Methods

A total of 85 Duroc x Landrace sows, distributed between the first and the sixth production cycle were used. Sows were kept in stalls from 0 to 35 days of gestation and then were moved to two dynamic groups, young (first and second cycle) and adult (multiparous). Dynamic courtyards (50 sows/pen), were partially slated and equipped with an automatic feeder (Nedap Velos de SERTIC S.A) and water nipple drinkers. Sows were fed the same feed during the entire gestation; 2080 Kcal NE/kg, 13.9% CP and 0.65% total lysine. The sows followed the farm feeding protocol based on recovering their body condition (0-35d) and their respective gestation feeding curve (35-90d). From day 35 to day 90 of gestation fed allowance was 2.5 and 2.1 kg/d for young and adult sows, respectively. Thereafter, three different balanced subgroups were fed for the last three gestation weeks regarding the feeding level: one following the protocol of the farm (STD) and the other two subgroups, supplemented with 0.5 and 1.0 kg/d. The duration of gestation (d) and farrowing (h) as well as the need of farrowing assistance were measured. Total born (TB), born alive (BA), stillborn (SB) and mummified (MM) were registered. Piglet's body weight was also registered at birth and after 18 days. For the statistical analysis, the sow was the experimental unit and the GLM type III and GENMOD procedure (SAS® version 9.3) was used.

Results

Supplementation (Table 1) did not affect the gestation length but increased farrowing length by 32% with +1,0 kg of extra feed (p=0.022). The number of TB was not affected, although non-significant, with +1,0 kg of extra feed, stillborn increased a 19.5% compared to STD and +0.5. Supplementing +0.5 kg brought about a non-significant increase (p=0.336) in piglet BBW of about 100g. Either the number of piglets weaned or the litter weight after 18 days of lactation were not affected by supplementation (p>0.10), which conversely produce a linear (p=0.062) increase of sow's back fat thickness (from 1.47 to 2.33 mm for STD and +1.0, respectively).

Table 1. Farrowing and lactation performance after feed supplementation in late gestation.

	STD	+0.5	+1.0	SEM	p
Nº sows	28	28	29		
Intake (*)	49.1^{c}	55.9^{b}	63.7^{a}	6.24	<0,001
Gestation (d)	115.1	114.9	114.7	0.74	0.324
Farrowing (h)	3.13^{ab}	2.80^{b}	3.70^{a}	1.203	0.022
Total born (TB)	12.97	12.51	12.75	3.018	0.841
Stillborn (SB)	0.87	0.86	1.04	1.052	0.774
BW of TB (kg)	1.65	1.75	1.72	0.257	0.336
BW of SB (kg)	1.67	1.76	1.74	0.259	0.349
Weaned	10.94	10.66	11.02	1.227	0.520
Litter BW 18d (kg)	63.5	61.2	63.2	10.32	0.429

* Feed intake from 90 to 111 days (g/kg $PV^{0.75}$/d); TB exclude mummies: Values without a common superscript are different.

Feeding +1.0 kg required more farrow assistance than STD or +0.5, especially with young sows; even though, broadly, adult sows needed more help than young (Table 2).

Table 2. Percentage of assisted sows during farrowing depending on age (young vs adults) and supplementation (STD, +0.5, +1.0).

% (of sows)	STD	+0.5	+1.0	Total
Young %	0 (14)	0(13)	31(13)	8(40) ^{x}
Adults %	14(14)	7(15)	19 (16)	14(45)^{y}
Total	7(28) ^{a}	4(28) ^{a}	24(29) ^{b}	10(85)

^{a,b} Values with different letters in the same row are significantly different (p<0.05).

The results will probably differ modifying the basal level of feeding or increasing prolificacy, although overfeeding along gestation bring about fatter sows, farrowing troubles, lower feed intake during lactation and economic losses (5).

Conclusion

The results suggest that adding +1.0 kg/d did not improve the BBW beyond supplementing at +0.5 kg/d; nevertheless, it increased farrowing length, the need for assistance and the number of stillborn.

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Comparing spring and summer performance of lactating sows fed with an equivalent amount of feed

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Introduction

Heat stress reduces performance in lactating sows and voluntary feed intake usually decreases when ambient temperatures exceed the thermoneutral zone (1). However, this feed intake reduction does not fully justify performance reduction usually observed in commercial conditions during the hot season (2). The study aims to quantify performance and energy partitioning of lactating sows fed with the same amount of feed in summer and spring.

Materials and Methods

Two batches (July vs April-May) of 25 lactating Hermitage (Large White x Landrace) sows from a commercial farm managed following a three-week management system were used. Sows were homogeneously distributed by cycle from one to sixth. The farrowing barn was equipped with natural ventilation and piglet's heating floor. After farrowing sows were manually fed twice a day a commercial lactation diet (3090 kcal ME/kg, 17.5% CP and 1.02% total lysine) following a pre-stablished curve; mean feed intake (kg/d) offered during spring (4.33±0.36) was equivalent to the "*ad lib*" registered in summer (4.34±0.66). At cross fostering (CF; day one of lactation) litters were equalized. Number of piglets and litter weight was measured at CF, d 7, d 14 post-farrowing and at weaning (21d). Sow's body weight (BW) and back fat thickness (BT) were measured at the entrance to the farrowing barn and at weaning. Sow BW just after farrowing was estimated using the equation published by Mallmann *et al*. (3) and the individual sow's body energy (NE), lipid and protein body mass were estimated using sow BW and BT and following Dourmad *et al* (4). Data was analyzed with ANOVA and GLM using the GLINMIX procedure of SAS V.9.3 (SAS Inst. Inc., Cary, NC).

Results and Discussion

The mean temperatures at the farrowing barn along the 21 lactation days were 22.4±0.59 and 27.6±1.29 and daily variation (max. minus min.) were 2.8±0.85 and 6.4±2.74 in spring and summer, respectively. Three sows did not finished lactation and were removed from the experiment.

At weaning litter size was unaffected by the season but litter BW (p=0.015), total litter BW gain (p=0.003) and individual piglet's BW (p=0.009) were higher in spring than in summer (Table 1). The main effect in litter BW seen at weaning was already obtained at 7 d of lactation (p<0.001); since sows had similar daily feed intake the litter behavior itself may help to explain the differences.

Table 1. Spring and summer comparison of litter performance after 21d of lactation.

	Spring	Summer	SD	*p*-value
Nº of sows	22	24		
Litter size CF	12.20	12.00	1.043	0.520
Litt. size Wean	10.76	10.13	1.052	0.142
Litt. BW at CF	19.71	20.13	3.693	0.716
Litt. BW7d	30.85	23.13	7.034	0.001
Litt. BW21d	63.3	52.6	13.66	0.015
Litter Gain21d	43.9	32.5	11.72	0.003
Piglet BW	5.87	5.16	0.843	0.009

CF, cross fostering, BW, body weight.

Lactating sows lost more BW (p=0.001), NE (p=0.001), body fat (p=0.004) and body protein (p=0.001) in spring than in summer, meanwhile BT losses were apparently unaffected by the season (Table 2).

Table 2. Sow's body weight (BW), back fat thickness (BT) and energy partitioning in the body of the sow.

	Spring	Summer	SD	*p*
BW_end_gestation	280.2	273.2	24.35	0.359
BW_farrowing	255.4	243.4	23.12	0.100
BW loss21d	25.7	10.4	11.80	0.001
BT farrowing	17.7	16.6	3.47	0.291
BT loss	2.86	2.75	1.508	0.820
Body Energy Farr.	737.8	687.4	87.94	0.071
NE loss (Mcal)	111.9	63.0	43.51	0.001
Body Fat Farr. (kg)	51.4	47.3	7.35	0.078
Body Fat Loss	9.25	5.87	3.530	0.004
Body Pro. Farr. (Kg)	40.0	38.4	3.91	0.173
Body Pro. Loss	3.45	0.87	1.972	0.001

BW, body weight; BT, back fat thickness; NE, net energy.

Conclusions

Providing an equivalent daily feed intake, both, litter performance and sow's tissue mobilization were lower in the hot season, suggesting that litter behavior may be involved. This effect may be caused by the litter apathy in the switch-on of lactation in summer.

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A nutritional emulsifier improves growing-finishing pig performance

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Introduction

Energy is a major cost factor in swine diets. Due to their high energy density, fats and oils are important energy sources in feed formulation. Improving the energy efficiency of these raw materials and feed is of much interest from an economical point of view. Nutritional emulsifiers can be used to improve energy digestibility and, thus, improve energy efficiency. This could result in more cost-effective diets without losing on performance. Previous studies on nutritional emulsifiers focused on the application of mixtures containing glycerol polyethylene glycol ricinolate (GPGR). Sun and Kim (2019), showed that GPGR is an agent that can be used to improve emulsification of fats in swine. In this study, it is hypothesized that GPGR as such, can also have a significant impact on the performance of swine and in energy reduced diets.

Materials and Methods

Two hundred and seventy pigs (n=270), mixed-sex PIC CA 25 x 410 sire, were allocated to three groups: i) control group (CG), where pigs had continuous access to a corn-cassava-soy based diet (2400 kcal/kg, 1.18% standard ileal digestible lysine (SID) in the first phase, 20-50 kg and 0.95% in the second phase, 50 kg and up) with 2.17% palm oil (refined) and ii) emulsifier group (EGR), where pigs had continuous access to a reduced energy (2350 kcal/kg) corn-cassava-soy diets with the same crude protein level (18%, first phase, and 16%, second phase) and amino acid level supplemented with a GPGR based nutritional emulsifier with a specific high hydrophilic-lipophilic balance (HLB), Excential Energy Plus (0.035%, Orffa Additives BV, Werkendam, The Netherlands). The third group (EGT) of pigs received the control diet supplemented with the GPGR based nutritional emulsifier. At the start of the trial, pigs (63 days of age, 17.4 kg on average) were divided over 18 pens (6 pens/treatment) each containing fifteen animals. After a 77-day trial period the following parameters were analyzed: body weight (BW, kg), average daily gain (ADG, g/day), average daily feed intake (ADFI, kg/pig), feed conversion ratio (FCR). Data were subject to ANOVA and mean comparison using Tukey's Test. SAS University edition was used in the analyses.

Results

Data is shown in table 1. The nutritional emulsifier had a statistically significant effect on BW and % pigs > 700 ADG when applied on top (EGT). The nutritional emulsifier had a near significant effect on FCR when applied on top (EGT).

Table 1. Effect of a nutritional emulsifier on production parameters of growing-finishing pigs.

Parameter	CG	EGR	EGT	P
BW (day 140)	87.39[a]	87.64[a]	94.04[b]	0.010
ADFI (kg/pig)	2.254	2.247	2.197	0.203
ADG (g/day)	923	903	990	0.104
FCR	2.438	2.489	2.218	0.059
% pigs > 700 ADG	10.0[a]	5.7[a]	32.7[b]	0.019

Superscripts indicate statistically significant differences ($p \leq 0.05$).

Conclusions and Discussion

The data suggests that the supplementation of a specific nutritional emulsifier positively impacts performance of growing-finishing pigs when applied on top. When energy reduced diets are supplemented with a specific nutritional emulsifier there is a cost-benefit, on the feed side, without losing on performance.
These results justify additional research in swine with GPGR-based nutritional emulsifiers.

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Enhancing colostrum consumption and piglet performance in suckling period using fermented potatoes protein supplementation in late gestating sows

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Introduction

Colostrum affects the growth and development of immunity in newborn piglets because colostrum is a rich source of energy and immunity. The nutritional status in late gestating sows influenced the colostrum in lactation period (1). Therefore, the objective of the present study was to determine the effect of fermented potatoes protein supplementation in late gestating sows on colostrum consumption and piglet performance in suckling period.

Materials and Methods

The study was carried out in a commercial swine herd in Thailand. A total of 95 sows were included in this study. Sows were allocated to two experimental groups: Control group, sows were fed a conventional gestation diet; and Lianol®50 group, sows were supplemented with 1.5 g/sow/day of Lianol®50. The feeding protocols were carried out from 7 days before predicted farrowing date until farrowing. The piglets were weighed at immediately after birth, Day 1, 3, 10, 17 and 21 after birth. The individual colostrum consumption of each piglet was estimated by a previously reported equation (Theil et al., 2014): Colostrum intake (gram) = -106 + 2.26WG + 200BWB + 0.111D - 1414WG/D + 0.0182WG/BWB; where WG is piglet weight gain over 24 h (gram), BWB is birth weight (kilogram), and D is the duration of colostrum suckling (min). All parameters were analyzed by using general linear mixed model procedure. The statistical model included treatment group, parity and interaction between treatment group and parity. Sow ID was included as random effect. Sow parity was classified into two groups including 1 and 2-7. $P < 0.05$ was regarded to be statistically significant. (SAS® program 9.2, Cary, NC, USA).

Results

On average, the colostrum consumption was 451.7 ± 167.2 grams. The colostrum consumption in Lianol®50 group sows (469.6 grams) was higher than Control group sows (431.5 grams, $P < 0.001$). Piglet performance in suckling period in control and Lianol®50 group sows were presented in Table 1. As can be seen from the table, Lianol®50 group sows have piglet birth weight and weight at Day 1 higer than Control group sows ($P < 0.01$). Moreover, weight at Day 17 and 21 after birth in Lianol®50 group sows have a tendency higher than Control group sows ($P < 0.1$).

Table 1. Effect of fermented potatoes protein supplementation on piglet performance (Least square mean).

Weight, kg	Control	Lianol®50	*P*
At birth	1.38 ± 0.37[b]	1.44 ± 0.36[a]	0.006
Day 1	1.49 ± 0.39[b]	1.56 ± 0.39[a]	0.004
Day 3	1.71 ± 0.46	1.75 ± 0.46	0.226
Day 10	3.02 ± 0.94	3.10 ± 0.87	0.204
Day 17	4.34 ± 1.56	4.51 ± 1.37	0.102
Day 21	5.05 ± 1.69	5.27 ± 1.59	0.055

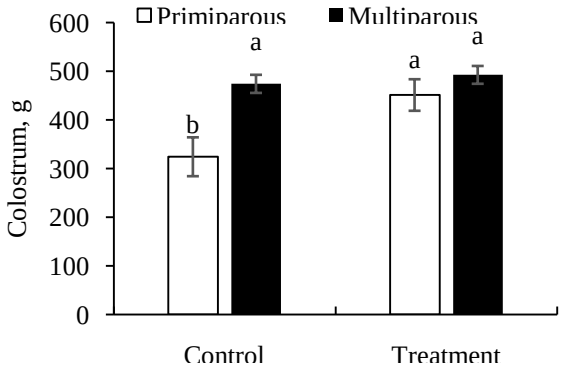

Figure 1. Piglet colostrum consumption in control and Lianol®50 groups by sow parity; [a, b] significant difference ($P < 0.05$)

The colostrum consumption in Control and Lianol®50 group by parity were presented in Figure 1. As can be seen from the figure, primiparous sows in Control group have the lowest colostrum consumption ($P < 0.05$) but did not differ significantly in multiparous sows.

Conclusions and Discussion

Fermented potatoes protein supplementation in late gestating sows increased colostrum comsumption and piglet birth weight and weight during suckling period.

Acknowledgement: Financial support for the present study was providing by Faculty of Veterinary Science and a grant for Development of New Faculty Staff Ratchadaphiseksomphot Endowment Fund, Chulalongkorn University (M. Nuntapaitoon).

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Improving colostrum composition using dietary fermented potato protein supplementation in late gestating sows

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Introduction

Piglets have high chance survival when they receive adequate colostrum (1). Because the colostrum has high immunoglobulin and energy for the first day of life. Increasing energy and protein intake in late gestating sow enhance fat and protein contents in colostrum (2, 3). Therefore, the objective of the present study was to determine the effect of fermented potatoes protein supplementation in late gestating sows on sow colostrum composition.

Materials and Methods

The study was carried out in a commercial swine herd in Thailand. A total of 95 sows were included in this study. Sows were allocated to two experimental groups: Control group, sows were fed a conventional gestation diet; and Lianol®50 group, sows were supplemented with 1.5 g/sow/day of Lianol®50 . The feeding protocols were carried out from 7 days before predicted farrowing date until farrowing (average 8.1 ±1.5 days). The colostrum samples were collected manually 0-1 h after onset of parturition. The all colostrum samples were filtered through gauze and were pooled and kept in a clean bottle (30 ml). The samples were centrifuged at 12,000 xg, 20 minutes at 4°C and were stored at -20°C until analysis. The concentration of immunoglobulin G (IgG) and insulin-like growth factor I (IGF-1) were determined using ELISA (Bethyl Laboratories Inc., Texas, USA) and the Mediagnost IGF-I ELISA E20 kit (Mediagnost Gesellschaft für Forschung und Herstellung von Diagnostika GmbH. Reutlingen, Germany), respectively. Colostrum composition were analyzed for fat, protein, lactose casein and dry matter concentration by infrared spectroscopy (MilkoScan FT2 instrument, Foss MilkoScan, Hillerød, Denmark). Descriptive statistics including mean, standard deviation and range of the data were calculated. All parameters were analyzed by using general linear model procedure by SAS (SAS® program 9.2, Cary, NC, USA). The statistical model included treatment group, parity and interaction between treatment group and parity. Sow parity was classified into two groups including 1 and 2-7. $P < 0.05$ was regarded to be statistically significant.

Results

Colostrum composition in Control and Lianol®50 group sows were presented in Table 1. As can be seen from the table, the colostral fat in Lianol®50 group sows have a tendency higher than Control group sows ($P < 0.1$).

Table 1. Effect of fermented potatoes protein supplementation on the colostrum composition (Least square mean)

Composition	Control	Lianol® 50	*P* value
Fat, g/100g	4.6	5.3	0.069
Protein, g/100g	18.7	17.6	0.167
Lactose, g/100g	2.2	2.2	0.796
Casein, g/100g	13.6	12.6	0.129
Dry matter, g/100g	25.0	24.7	0.773
IGF-1, ng/ml	145.4	133.3	0.688
IgG, mg/ml	51.9	55.1	0.518

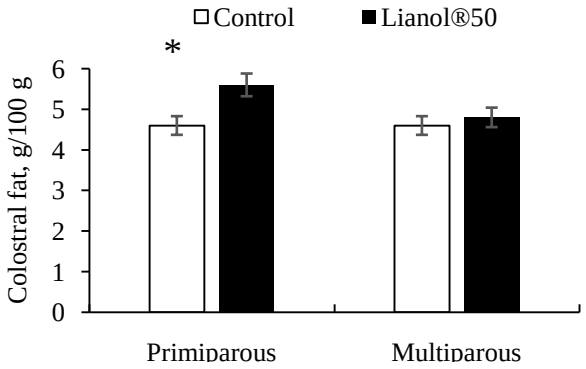

Figure 1 The colostral fat in in Control and Lianol®50 group sows by parity; *significant difference ($P < 0.1$).

As can be seen from the figure, the colostral fat in primiparous sows in Lianol®50 group sows have a tendency higher than Control group sows ($P = 0.086$).

Conclusions and Discussion

Fermented potatoes protein supplementation in late gestating sows increased colostral fat, especially in primiparous sows.

Acknowledgement

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Dietary fermented potato protein supplementation in late gestating sows reduces sow relative backfat loss at weaning

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Introduction

Maintaining optimal body weight during late gestation and lactation influenced sow and piglet performance in next parity (1, 2). Moreover, high backfat loss before weaning increase weaning-to-oestrus interval, culling rate and decrease litter size in sows (3). Therefore, the objective of the present study was to determine the effect of fermented potatoes protein supplementation in late gestating sows on sow backfat thickness and relative backfat loss during lactation period.

Materials and Methods

The study was carried out in a commercial swine herd in Thailand. A total of 95 sows were included in this study. Sows were allocated to two experimental groups: Control group, sows were fed a conventional gestation diet; and Lianol®50 group, sows were supplemented with 1.5 g/sow/day of Lianol®50. The feeding protocols were carried out from 7 days before predicted farrowing date until farrowing. The backfat thickness of the sows were measured at the level of the last rib at about 6-8 cm from the midline using A mode ultrasonography (Renco Lean-Meater®, Minneapolis, MN., USA.). The backfat measurement was performed in each sow at farrowing, 7 day after farrowing and at weaning. The backfat loss is defined as the difference between backfat thickness at farrowing and 7 day after farrowing and the difference between backfat thickness at farrowing at weaning in each sow. The relative loss of backfat (%) is defined as the backfat loss (mm) divided by backfat thickness at farrowing and multiplied by 100. Descriptive statistics including mean, standard deviation and range of the data were calculated. All parameters were analyzed by using general linear model procedure by SAS (SAS® program 9.2, Cary, NC, USA). The statistical model included treatment group, parity and interaction between treatment group and parity. Sow parity was classified into two groups including 1 and 2-7. $P < 0.05$ was regarded to be statistically significant.

Results

On average, the backfat thickness at farrowing, 7 days after farrowing and weaning were 13.6 ± 1.3, 10.1 ± 1.6 and 13.1 ± 2.0 mm, respectively. The backfat thickness and the relative backfat loss in lactation period in control and Lianol®50 group sows were presented in Table 1. The Lianol®50 group sows have the relative backfat loss at 7 days after farrowing and at weaning lower than Control group sow (Table 1).

Table 1. Effect of fermented potatoes protein supplementation on the backfat thickness and the relative backfat loss (Least square mean).

Parameters	Control	Lianol®50
Backfat, mm		
Farrowing	13.7	13.6
7 days after farrowing	9.8	10.3
Weaning	12.5	13.5
Relative backfat loss, %		
7 days after farrowing	27.7	24.1
Weaning	6.8	-0.3

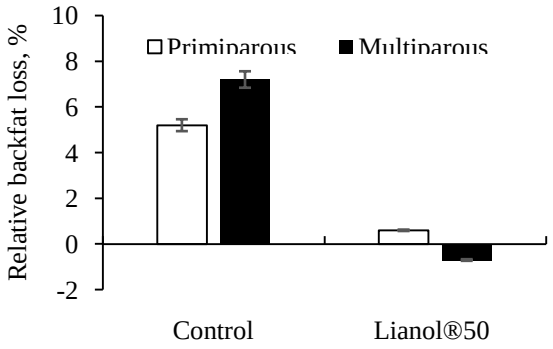

Figure 1. The relative backfat loss at weaning in primiparous and multiparous sows by treatment group

As can be seen from the figure, in multiparous sows, the relative backfat loss at weaning in Lianol®50 group sows have a tendency lower than Control group sows ($P = 0.121$).

Conclusions and Discussion

Fermented potatoes protein supplementation in late gestating sows reduce the relative backfat loss at weaning in sows.

Acknowledgement

Financial support for the present study was providing by Faculty of Veterinary Science and a grant for Development of New Faculty Staff Ratchadaphiseksomphot Endowment Fund, Chula longkorn University (M. Nuntapaitoon).

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Development of novel edible toys loaded with iron to prevent iron deficiency anemia in weaned pigs

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Introduction

The main nutritional deficiency of lactating and weaned pigs in intensive breeding systems is iron deficiency anemia (1). To prevent it, an intramuscular dextran iron dose of 200 mg is used during the first days of life. However, it has been lately described that this dose is not being effective, finding pigs anemic, deficient and with iron depleted deposits at weaning (2). Therefore, alternative supplementation strategies are needed. The objective of the present study was to develop and characterize edible toys as oral iron supplements, and to assess their effect on feeding behavior and iron nutrition status of weaned pigs.

Materials and Methods

Edible toys (ET) were prepared based on sodium alginate at 2% w/v in distilled water and dried whey at 40% w/v, plus 1% w/v of heme iron and 1% w/v of ferrous sulfate. Three types of ET were manufactured, 1) control (ETC): without sweetener, 2) ET1: with 15% w/v of sucrose, and 3) ET2: with 0.03% w/v of Sucram® (98% sodium saccharin, 1% neoesperidin dihydrochalcone and 1% maltol). ET were characterized by digital photography, dimensions, proximal chemical analysis and iron content. Behavioral tests were conducted to determine the pigs acceptability and preference for ET (Figure 1). For this, 12 pairs of weaned male and female pigs (21-day-old, [Large White x Landrace] x Pietrain) were randomly distributed to the three treatment groups: ETC, ET1 and ET2. Acceptability was determined during three consecutive days, and preference during one day. In another study with 16 weaned pigs, we determined the effect of consumption of edible toys with iron (FeET) and without iron (ET), delivered for 16 days, on some biomarkers of iron status at days 1 and 16 after weaning. All the pigs were fed a diet of 200 ppm iron. The data was analyzed with a mixed effect two-way ANOVA model followed by Tukey adjusted t-test ($p<0.05$) (9.4 SAS® Institute Inc., Cary, NC, USA).

Results

The three types of ET proposed were obtained. They have similar physical characteristics, being oval in shape, with a diameter of 3.8 cm and a weight of 9.7 g. They were brown in color (Figure 1). The ET were mainly composed of carbohydrates (71-78%) and protein (9-12%, dry basis). The iron content was similar in all the ET (2.5-2.7 mg/g).
No significant differences were observed in ET acceptability ($P = 0.2462$). However, ET1 showed a lower consumption and preference of 17%, as compared to ETC and ET2 that showed a higher consumption and a preference, close to 40%.
The effect of ETs on iron status of pigs is presented in Table 1. The time factor was potent on the evolution of the pigs' iron status, with significant differences found in all biomarkers after supplementation. This indicates that supplementation was effective in improving the iron status of pigs, especially for FeET group, where 90% of the pigs had an optimal iron status. Unlike the ET group, where 80% of the pigs had their iron deposits depleted. When comparing between treatments at the end of supplementation, the ET group showed lower values of the biomarkers RBC, Ht, serum iron and serum ferritin than the FeET group.

Conclusions and Discussion

It is possible to vehicle iron in edible toys, which have high acceptability and consumption by weaned pigs. Its use as a supplement to the weaning diet may improve the iron status of pigs, preventing iron deficiency anemia.

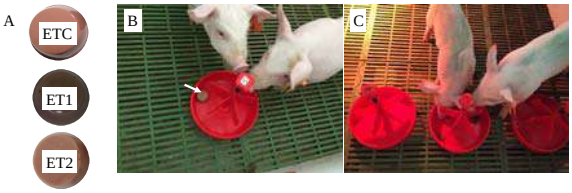

Figure 1. Aspects of ET (A). Images of the acceptability (B) and preference (C) tests. The white arrow indicates the ETs in the feeders.

Table 1. Effect of supplementation with ETs on iron status of pigs.

Biomarkets and cut-off	ET		FeET		P time	P group
	D1	D16	D1	D16		
RBC (<5.3)	6.1	6.3	6.1	7.5	0.004	0.001
Hb (<9)	8.5	9.8	8.8	10.2	0.156	0.001
MCV (<50)	51.8	53.3	50.5	53.9	0.825	0.015
Ht (<32)	32.4	34.2	32.8	37.9	0.000	0.001
SFe (<30)	26.4	65.8	28.6	120.6	0.001	0.001
TIBC (>640)	595	389	564	316	0.135	0.001
SF (<12)	7.7	11.2	7.8	18.5	0.001	0.002

RBC: red blood cell, Hb: hemoglobin, MCV: median corpuscular volume, SFe: serum iron, TIBC: total iron binding capacity, SF: serum ferritin.

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Evaluation of an injectable macro and trace minerals supplement on reproductive performances in field Brazilian conditions

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Introduction

Late pregnancy and lactation in sows are periods with high metabolic requirements, all the more with genetic and herd management trends toward increased productivity. It can induce oxidative stress and consequently reduce reproductive performances. Objective of this study was to assess possible improvement of sows reproductive performances by implementing an injectable treatment program based on essential macrominerals and trace minerals in field Brazilian conditions.

Materials and Methods

The trial was performed in a farrow to finish farm owning 370 sows. A total of 59 lactating sows from 5 successive batches were allocated between a tested group (T) and a control group (C).

Each sow from the T group received the tested product twice, at weaning and one month before expected next farrowing (5 ml by intramuscular route for each injection). Sows from the C group did not receive any injectable mineral supplement. The tested product contains phosphorus both as organic (sodium glycerophosphate) and inorganic (sodium phosphate) forms, potassium, magnesium, copper and selenium (Fosfosal®, Virbac).

All sows were followed between weaning and next farrowing by recording the delay between weaning and fertile artificial insemination (AI) and the number of born alive per litter for farrowing sows. Conception and farrowing rates, delay between weaning and positive AI and number of born alive per litter were respectively compared between groups by Fisher exact test, Kruskal-Wallis test and t test.

Results

No general neither local side effect were observed after injection of the tested product to all sows of T group.

Conception and farrowing rates were numerically higher in T group. The mean delay from weaning to positive AI was 3 days shorter in T group. The median was the same in both groups but the range was much larger in C group, reflecting return to oestrus of some sows. For this criteria, difference between groups was close to significance (p=0.08). The mean number of born alive per litter was numerically higher in T group than in C group (+ 0.8), close to significance (p=0.1).

Table 1. Reproductive performances.

		T	C
Conception rate	%	96.4	90.3
Weaning to positive AI (days)	Mean ± SD Median (Range)	6 ± 7 4 (3-25)	9 ± 11 4 (4-53)
Farrowing rate	%	96.4	83.9
Born alive per litter	Mean ± SD	13.7 ± 1.8	12.9 ± 3.0

Discussion and conclusion

Positive trends on sows return to oestrus after weaning, successful pregnancy and prolificacy were observed when the tested product was administered twice during strategic stages (weaning and end of pregnancy). Thus focused injectable macro and trace minerals supplementation could improve reproductive performances. Particularly, selenium is known as being part of the antioxidant system and may contribute to recovery of the oxidative status equilibrium (1).

As no statistical significance between groups could be achieved, probably due to limited number of included sows, these preliminary results should be confirmed at a wider scale.

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Field trial of a nutritional supplement in piglets at the time of weaning

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Introduction

Weaning is a critical period for piglets due to change of nutrition, housing and animal grouping. Particularly digestive disorders can occur after weaning. Objective of the present study in piglets was to test an oral effervescent powder containing lactose, whey powder, rice flour, carob powder, vitamin E, selenomethionine and *Enterococcus faecium* NCIMB 10415 (Enerlyte® plus, Virbac).

Materials and Methods

This study was performed in a farrow to finish farm owning 400 sows of DanBred origin in Spain.

A palatability test was done by comparing consumption of either pure water or water supplemented with Enerlyte® plus on weaned piglets aged 21 days. Respectively 67 piglets from 5 pens in tested (T) group and 49 piglets from 4 pens in control (C) group were included in the palatability test.

A zootechnical test was done on piglets aged 24 days in average. One hundred thirty piglets were allocated between T and C groups (5 pens per group). These piglets were issued from sows of parities 2 to 5, with litter size between 10 to 16 piglets per litter. Piglets received Enerlyte® plus in T group from D1 (start of trial) to D5. Piglets were individually identified by ear-tag and weighed thrice on D1, D8 and D32 (end of transition).

In both palatability and zootechnical tests, Enerlyte® plus was diluted in water (100 g/2 l) and administered twice per day for 2 h per distribution in T group (100 g/10 piglets/day). The same volume per piglet of non supplemented water was distributed in C group. In both groups, water was administered in one drinker per pen and feeding was identical.

In the zootechnical test, body weights and average daily gains (ADG) were compared between groups according to the t test.

Results

In the palatability test, consumption of supplemented water in T group was complete within the 2 hours following first distribution whereas it reached only 75% of the non supplemented water in C group.

In the zootechnical test, mean body weight on D32 was significantly higher in T group than in C group. The ADGs between D8 and D32, and between D1 and D32 were also significantly higher in T group (Table 1).

Table 1. Body weights (kg) and ADG (g/d) (Mean ± SD).

	T	C
Weight (D1)	6.96 ± 1.52	6.69 ± 1.16
Weight (D8)	7.31 ± 1.57	6.90 ± 1.15
Weight (D32)	15.09 [a] ± 2.60	13.75 [b] ± 2.04
ADG D1/D8	47.6 ± 58.3	30.0 ± 52.5
ADG D8/D32	320.2 [a] ± 57.3	286.0 [b] ± 50.0
ADG D1/D32	259.6 [a] ± 48.4	227.7 [c] ± 41.4

[a], [b]:Significant difference between groups ($p \leq 0.005$)
[a], [c]:Significant difference between groups ($p \leq 0.0001$)

Discussion and conclusion

Palatability of the tested product was confirmed.

Growth was significantly increased in T group from D8. A numerical increase was also noticed between D1 and D8, reflected by a lower rate of piglets loosing weight during that week (12% in T group vs 24% in C group). Among the components of the nutritional supplement tested, *Enterococcus faecium* NCIMB 10415 has been shown as enhancing absorption of glucose by the jejunum of pigs, which could increase absorption of nutrients and prevent diarrhea by increased water absorption (1). Moreover effect of that probiotic on growth in nursery has been previously reported (2). In present study, the average increase of weight gain in the T group during the transition period was around + 1 kg. This should be put in perspective with general rule from textbooks converting 1 kg difference at end of transition into 3 kg at finishing (3).

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Microbiome changes in the fundic region, pars oesophageal, and gastric content of nursery pigs with gastric ulcers

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Introduction

A balanced gastrointestinal microbiota is associated with health and well-being in pigs (1). Improved understanding of the gastric microbiome may provide an important insight into the mechanism of gastric ulceration development in pigs. Therefore, in this study, we assessed variations in the gastric microbiome composition in nursery pigs with and without pars oesophageal ulcers (POU).

Materials and Methods

In total, 39 commercial nursery pigs DanBred (Duroc × Landrace × Yorkshire), 15 with POU and 24 with healthy stomachs were sampled at 10 weeks of age. All nursery pigs shared the same all-in/all-out production cycle, farm management, and were fed the same commercial pelleted feed ad libitum.

Samples were collected at three gastric locations: Swab samples from fundic mucosa and pars oesophagea mucosa and a spoon sample of gastric content.

The prokaryotic microbiome at the three sampling sites was determined by 16S rRNA gene (V3-region) amplicon sequencing as described previously (2).

Results

Fundic mucosa, pars oesophagea mucosa, and gastric content in all nursery pigs each presented a different microbial community structure based on weighted and unweighted UniFrac analysis, ($p = < 0.01$), Figure 1.

Furthermore, the prokaryotic microbiome showed distinct signatures between nursery pigs with and without POU assessed by unweighted UniFrac (PERMANOVA $P = < 0.006$; $F = < 2.17$), which was driven by higher relative abundance of specific bacterial taxa, Figure 2.

Nursery pigs with POU presented a loss in microbial diversity in the fundic mucosa and gastric content (t-test with Monte Carlo simulations; $p<0.05$).

The pars oesophageal mucosa of nursery pigs with POU presented a trend in microbial diversity loss (t-test with Monte Carlo simulations; p 0.057).

Conclusions and Discussion

We have demonstrated that the microbial composition of the stomach in nursery pigs with POU is significantly different from those without POU. However, further research is required to assess if changes in the microbial composition in nursery pigs with POU can influence gut health or productivity. Similarly, whether those microbial changes are maintained in other gut segments and the feces.

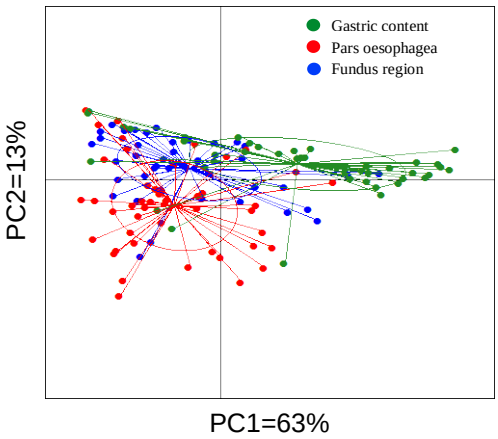

Figure 1. The prokaryotic microbiome of gastric content, fundic mucosa, and pars oesophageal mucosa differs in composition (Principle Coordinate Analysis-based depiction of unweighted Unifrac distance metrics).

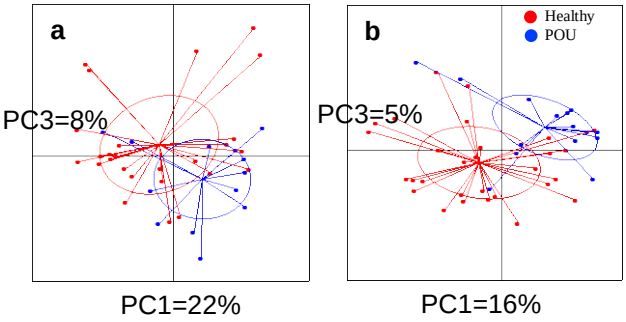

Figure 2. Differences in the prokaryotic microbiome of fundic mucosa (**a**) and pars oesophageal mucosa (**b**) differs in composition in nursery pigs with and without POU unweighted Unifrac distance metrics

Acknowledgments

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Low birth weight piglets show reduced mortality and antibiotic treatment with Piglet Protector®

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Introduction

Mortality in suckling piglets is an important issue in terms of animal welfare and economic return. Literatures show the average mortality of piglets in suckling period is 14% and the most critical time is within the first 24 hours after birth. Several factors are influencing the mortality, birth weight and vitality of piglets are part of it (2). Low birth weight piglets show higher mortality rates and less weight gain (1). Special support of these piglets is therefore necessary.

The main ingredients in Piglet Protector® are immunoglobulins, probiotics (Bacillus spp. and lactic acid bacteria) and medium-chain-fatty acids. Piglet Protector® supports piglets in the first days of life, which is shown in several field trials (own research). The following trial observes only low birth weight piglets

Materials and Methods

The trial was conducted in the Philippines in 2018 in a commercial sow farm. 20 sows and their litters were included. Each litter was divided into trial and control group. Only piglets with a birth weight between 0.7 kg and 1.0 kg were observed. The parity of the sows was one, two or three and the lactation period lasted 28 to 30 days. Piglet Protector® was given to the trial piglets within 6 to 12 hours after birth, a second dose was given after another 12 hours. Piglets with diarrhea were treated with antibiotics in both groups. Measured parameters were birth and weaning weight, mortality and the treatment with antibiotics.

All data were subjected to statistical analysis using SPSS, Vers. 24 (IBM SPSS). After testing normal distribution of the data, they were analysed by Oneway ANOVA. Differences at $p \leq 0.05$ were considered as significant.

Results

Despite the lower average birth weight of the trial group, the weaning weight of the trial piglets was significantly higher (Tab. 1). That is also significantly better in average daily weight gain (244g vs. 220g, p=0.000).

The mortality of the piglets in the trial group was reduced by more than two thirds as compared to the control group (Fig. 1). Nearly the same result was observed with regard to antibiotic treatments (4.5% vs. 15.0%).

Table 1. Daily weight gain of underweight piglets

	Trial	Control	p-value (T-test)
Av. parity of the sow	2.2	2.2	
No. of piglets	44	40	
Av. birth weight (kg)	0.828[a]	0.875[b]	p=0.01
Av. weaning weight (kg)	7.947[b]	7.256[a]	p=0.000
Av. weaning age (days)	29.2	29.1	p=0.58
Av. daily weight gain (kg)	0.244[b]	0.220[a]	p=0.000

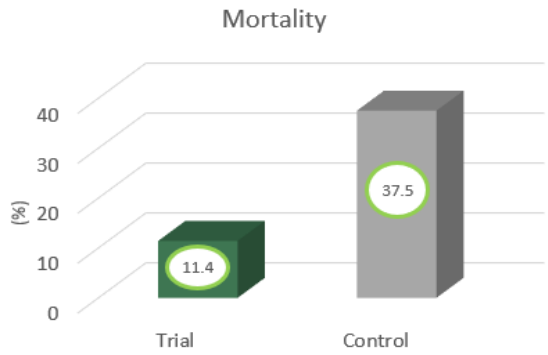

Figure 1. Piglet mortality during the suckling period

Conclusions and Discussion

Piglet Protector® supports underweight piglets to – establish a better start into life. The early and fast colonization with beneficial mircroorganisms in the gut can reduce antibiotic treatment due to less intestinal disturbances. A healthy gut is crucial for the resorption of nutrients and therefore a sufficient weight gain. The energy supply through medium chained fatty acids improves the vitality of the piglets, giving them a better chance to reach more colostrum. It improves the immune system and promotes the young animal in critical phases.

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Effect of metal methionine hydroxy chelate supplementation on sow and litter performance in comparison with other sources of trace minerals

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Introduction

With intensive genetic selection for hyperprolific sows and the continuous improvement of management strategies, the average number of pigs weaned per sow in the EU and US has increased considerably in the past decade. However, the increased litter size has led to a higher percentage of low birth weight piglets within litter (1), which could lead to high pre-weaning mortality (2). Additionally, sow culling rate has been maintained at a high level, ranging from 42 to 49% from 2008 to 2017 in US (3), which may be driven by improper nutrition and poor management of gilts and sows. Therefore, the objective of this study is to investigate if supplementation of metal methionine hydroxy analogue chelate at reduced inclusion level could improve sow and litter performance in large scale commercial sow farms.

Materials and Methods

Three dietary treatments were used in this study: 1) 100 ppm Zn as ZnO, 25 ppm Cu as $CuSO_4$ and 45 ppm Mn as $MnSO_4$ (**ITM**); 2) 100 ppm Zn as Zn proteinate or Zn amino acid complex, 25 ppm Cu as Cu proteinate or Cu amino acid complex and 45 ppm Mn as Mn proteinate or Mn amino acid complex (**OTM**); 3) 50 ppm Zn as Zn methionine hydroxy analogue chelate (Zn-MHAC) (MINTREX® Zn, Novus International, Inc., St. Charles, MO, USA), 10 ppm Cu as Cu-MHAC and 20 ppm Mn as Mn-MHAC (**MMHAC**). A total of 82 commercial sow farms in Spain were randomly allotted to 1 of 3 dietary treatments, with 28, 26 and 28 sow farms in ITM, OTM and MMHAC treatments, respectively. Average number of sows on each farm in ITM, OTM and MMHAC were 1505, 1547 and 1546, respectively. Breed of sows (Topigs, PIC, Hypor and Danbred) were equally distributed in 3 dietary treatments. All sow farms used similar gestation and lactation housing systems. The study was conducted from January 1, 2016 to December 31, 2017.

Sow performance (Table 1) and litter performance (Table 2) were recorded and the average values for each parameter during 2 years for each farm were considered as replicates for each treatment.

The data were analyzed using GLIMMIX procedure of SAS® 9.4. POISSON distribution analysis was used for number of liveborn, dead, total born and weaned piglets, whereas BINOMIAL distribution analysis was used for farrowing rate, replacement rate, mortality rate, culling rate due to locomotion, sow retention rate till parity 3 and pre-weaning mortality rate.

Results

MMHAC significantly reduced sow mortality rate ($P < 0.01$; Table 1) and increased sow retention rate till parity 3 ($P < 0.01$), compared with sows fed ITM and OTM. Similarly, sows fed MMHAC significantly improved litter performance by reducing number of dead born piglets ($P < 0.01$; Table 2) and increasing number of weaned piglets ($P < 0.01$), compared with those fed ITM and OTM.

Table 1. Effect of trace mineral sources during gestation and lactation on sow performance

Items, %	ITM	OTM	M-MHAC	SEM	*P*-value
Farrowing rate	86.49b	87.24ab	87.44a	0.18	<0.01
Replacement rate	48.55a	46.14b	46.00b	0.27	<0.01
Mortality rate	8.48a	7.69b	7.20c	0.14	<0.01
Locomotion culling rate	18.61a	14.93b	14.85b	0.20	<0.01
Retention rate till P3	67.18c	70.69a	73.93b	0.25	<0.01

Table 2. Effect of maternal feeding of sources of trace minerals on litter performance

Items	ITM	OTM	MMHAC	SEM	*P*-value
Total born	14.36	14.35	14.36	0.03	0.99
Dead born	1.23a	0.81b	0.72c	0.02	<0.01
Live born	13.02b	13.41a	13.50a	0.04	<0.01
Weaned	11.53c	11.87b	12.12a	0.04	<0.01
Pre-weaning mortality, %	11.42	11.43	10.18	2.13	0.85

Conclusions and Discussion

MMHAC at about half inclusion levels were shown to exert several advantages over other organic trace minerals and inorganic trace minerals, in terms of improving sow retention rate, reducing sow mortallity rate and improving number of weaned piglets. The results indicate that MMHAC supplementation could further optimize sow reproductive performance and ensure hyperprolific sows could realize their genetic potential.

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Use of Provenia CF-Z in weaned piglets: results from a field trial in South-Europe

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Introduction

The early development of piglets depends directly on their health and nutrition, and it is key for their lifetime performance. Intestinal challenges are the main cause of performance losses during the post-weaning period. ZnO inhibits growth of certain pathogenic micro-organisms in the gut after weaning (1). Still there are some other strategies available. Benzoic acid is an organic acid that modify the intracellular pH of gut bacteria and shifts gut bacterial profile by minimizing pathogenic bacteria (2). When embedded in a fat matrix, it is enabled to be slowly released throughout the intestine. The objective of the trial was to assess the efficacy of Provenia CF-Z a blend of protected benzoic acid (PBA) on piglets' performance and health under field conditions.

Materials and Methods

A trial was run in 4 farms and piglets were fed a diet with protected benzoic acid (PBA; 2.5kg/ton) or a control diet containing ZnO at 2500 ppm. All the farms in the trial were in Spain with similar size and nutrition program (no antibiotics in feed). All of them used ZnO during the first 10 days post-weaning regularly. In total, 2608 piglets were fed with ZnO and 2618 with PBA. Feeds were iso-nutritional and only differ in the product tested for creep-feeding (7d-old up to 3-7 days post weaning) and pre-starter feed (from weaning (28d) to 6 weeks life). Piglets were allocated in pens and growth was monitored for the whole period after weaning: feed intake (FI), average daily gain (ADG), mortality, incidence of disease and medication costs. Fresh feces for each one of the farms (n=8) were taken at the end of the trial and bacterial DNA was isolated and extracted with FastDNA SPIN Kit for Soil (MP Biomedicals, USA) following the manufacturer's instructions. The V3-V4 region of the 16S rRNA gene was amplified (3) and sequenced using the Illumina MisSeq platform 300x2bp. The libraries were prepared using the standard protocol for MiSeq Reagent Kit V3 and sequenced on MiSeq platform (Illumina Inc., USA). For the bioinformatics analysis, the DADA2 pipeline was used (4). Performance data was analyzed using SAS Software 9.4 and microbiota results analysis were carried in R v3.6 using phyloseq and Vegan packages.

Results

Piglets fed diet containing PBA showed higher ADG and lower FCR in all the farms compared with ZnO piglets and there were not differences on FI. There was also a reduction on mortality, incidence of diarrhea and medication costs (Table 1). The alpha-diversity plot demonstrated a higher microbial diversity in the PBA group compared to the ZnO group. Differences were statistically significant for the Shannon and Simpson indices (p value=0.0008 and 0.006). For the beta diversity (Bray Curtis matrix), there were no distinct cluster for the treatment factor in the NMDS plot. The Adonis test shows that there were differences, in bacterial composition between treatment (p=0.079, R^2=0.034), farms (p=0.001, R^2=0.1287) and their interaction (p=0.002, R^2=0. 0820). At Phylum level, the PBA group showed a higher abundance of *Fibrobacteres* compared to the ZnO group (p= 0.041)

Table 1. ADG, FI, FCR, mortality, medication costs and incidence of diarrhea of piglets receiving PBA or ZnO for each one of the farms included in the trial.

GROUP/ FARM		ADG g/pig/d	FI g/pig/d	FCR	Mortality, %	Medication, €/pig	Diarrhea, n/100
Zn0	1	385b	510	1.33[a]	2.08[a]	0.50[a]	5[a]
	2	394b	573.7	1.46[a]	2.47[a]	0.40[a]	7[a]
	3	395b	550	1.39[a]	2.00[a]	0.30[a]	5[a]
	4	386b	550	1.42[a]	2.25[a]	0.35[a]	8[a]
PBA	1	395a	514	1.30[b]	1.72[b]	0.20[b]	2[b]
	2	404a	577.5	1.43[b]	1.74[b]	0.18[b]	3[b]
	3	408a	561	1.38[b]	1.18[b]	0.25[b]	2[b]
	4	395a	545	1.38[b]	1.43[b]	0.20[a]	5[b]
P value		<0.01	NS	<0.05	<0.01	<0.01	<0.05

Conclusions and Discussion

The use of PBA influenced the microbial profile of weaned piglets by increasing the alpha diversity, that is considered as marker for a more balanced, healthier and more mature microbiota (5) and by favouring the proliferation of known beneficial bacteria, which are related with the metabolism of polysaccharides. This finding on the faecal microbial profile can contribute to explain the higher ADG and lower FCR, incidence of diarrhea and mortality observed in piglets supplemented with PBA.

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Short-term effects of dietary nucleotide supplementation on small intestinal mucosa integrity and hematological parameters in newly weaned pigs

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Introduction

Currently weaning remains a stressor period for the piglet, in which the food consumption decrease during the first hours after weaning. The absence of enteral nutrients results in alterations of architecture and function of small intestine (1). The intestinal epithelial cells need nutrients to support their function and metabolism (2); for the enterocyte, nucleotides represent a major nutriment. These molecules play a major role in almost every biological process (3). Nucleotides are semiessential nutrients for tissue with high cell turnover or limited capacity for *de novo* synthesis such as intestinal epithelium and immune cells (4) which depend on the dietary support; however, post-weaning diets are nucleotide deficient. Nucleotide demands increase also in stress, tissue with rapid growth, intestinal injure and diarrhea. In this regard, nucleotides are conditionally essential nutrients to newly weaning piglets that undergo rapid growth of tissue and organ systems, process that are dependent on availability of nucleic acids and ATP energy, whose synthesis depends on availability of nucleotides (5). The aim of the present study was to assess the impact of specific nucleotides on the intestinal mucosa integrity and on selected markers of health status of piglet after weaning.

Materials and Methods

Sixty piglets weaned at 21 d were randomly assigned to a post-weaning diet, base wheat-soybean meal supplemented with 0% (T-0; n=20); 500; (T-500; n =20), 1000 (T-1000; n =20) ppm of nucleotides from a yeast extract contributing 5' IMP, 5'GMP at 5.0 % and 5.5 % respectively (Phileo by Lesaffre). At 0, 7 and 14 d post weaning 3 piglets from each treatment were sacrificed. Samples of jejunum were processed to histologic analysis. The height of 30 villus and crypt depth was measured using an optic microscope (Motic B3). Blood samples were collected (0800h) and selected hematological parameters, white blood cell (WBC), lymphocyte (Ly); red blood cell (RBC) and hemoglobin (Hb), were analyzed with an automated hematology analyzer Coulter AcT 5. Statistical analyses were performed using an ANOVA on a linear model and treatment means were compared with a Tukey test, JMP (SAS, 2012).

Results

The data showed significant differences between treatments in the length of the intestinal villus and crypt depth (P <0.01) as well as in the hematological parameters, table1

Table 1. Histological and hematological parameters of early-weaned piglets fed with different levels of nucleotides

	Nucleotides-supplemented diet		
Parameter	T-0	T-500	T-1000
WBC-10^3/µL	14.3±0.7^a	12.4± 0.7^a	16.0± 0.7^b
Ly-10^3/µL	8.7±0.5^a	7.0 ±0.5^a	10.8 ±0.5^b
RBC 10^6/µL	6.78±0.1^a	6.52±0.1^a	6.40±0.6^a
Hb-g/dL	10.76±0.3^a	11.0±0.1^a	11.1±0.1^a
Jejunum (µm)			
Villi height	260.8± 16^a	319.5±22^b	474.0±16^c
Crypts depth	188±19^a	170.2±16^b	147.32±16^c

Values are expressed as mean ± SE. Different letter in the same row indicate significantly different (P>0.01).

Conclusions and Discussion

In this study, nucleotide supplementation increased markedly the villi height in piglets. There was 81.8% of increase in piglets that consumed T-1000 diet compared to T-0 diet, and 22.5% in animals that received T-500 diet. Results that coincide with others in which dietary nucleotides has favorable effects on intestinal health. Hematological examination may contribute to the detection of some changes in health and physiological status, which may not be apparent during physical examination. Nucleotide supplementation seems to have a favorable effect on selected hematological markers. In conclusion, during the critical transition phase of weaning, nucleotides can be a suitable ingredient that helps to reduce the intestinal mucosa injure and improve the physiological and the health status of the piglet.

Acknowledgments

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Natural alternatives as growth promoters, on the productive yield of weaned pigs at 28 days of age

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Introduction

Antibiotics have been used in animal feed as growth promoters (AGP) (Heo *et al*., 2013), playing an important role in the growth and development of the pig industry. But concerns about residues of these compounds and the development of bacterial resistance have led to their regulation and prohibition (Begum *et al*., 2015). Therefore, both the industrial and scientific sectors have focused efforts on finding and developing new alternatives to the use of AGP. The most widely researched alternatives of natural origin to replace (totally or partially) to AGP, include probiotics (Herrera, 2015); acidifiers (Suiryanrayna & Ramana, 2015) and plant extracts (Cho and Kim, 2015). Given the high consumer demand for safe pork, the inclusion of alternative food additives instead of antibiotics in pig diets, is necessary to support profitable and sustainable pig production (Zimmermann *et al.*, 2016). Therefore, the aim was to evaluate natural alternatives as growth promoters, in the productive yield of weaned pigs at 28 days of age.

Materials and Methods

75 piglets (females and males) were used commercial terminal crossing, weaned at 28 days of age with an approximate weight of 7.8 ± 0.4 kg. All animals were housed in groups of 5 animals and each group randomized to one of 5 diets: Control Diet (CD): Balanced food without antimicrobial; Diet 2 (D2): CD with antibiotic (Zinc Bacitracin) (350 ppm); Diet 3 (D3): CD with *Bacillus subtilis* pb6 (PTA 6737) (50 ppm of food. 10^8 CFU); Diet 4 (D4): CD with oregano essential oil (*Lippia origanoides*) (AEO) (150 ppm); Diet 5 (D5): CD with organic acid (AO), mixture of acids: citric, fumaric, benzoic, sorbic, ascorbic, lactic and sodium butyrate (4ppm). Production data collection were taken on days 1 and 30 after weaning. The quantity of food offered and rejected to calculate total consumption, daily weight gain (DWG) and final weight (FW) was recorded; In addition, food conversion (FC) was calculated for these same dates.

Results

The control diet (CD) animals had the lowest values for the variables FW and DWG. On the other hand, piglets belonging to the antibiotic alternative antimicrobial groups had the highest FW, DWG and higher feed consumption (D3> D4> D5, respectively) ($P < 0.05$) compared to pigs in group D2 (antibiotic). With this in mind, the FC parameter for pigs of D3> D4> D5 also presented the best values (lowest FC), compared to pigs in D2 ($P < 0.05$).

Discussion and Conclusions

D3 pigs had better production rates, with an increase in DWG and a decrease in FC, which is consistent with Michiels *et al.* (2016), who added *B. subtilis* in piglet feed, improving FC. This, explained by the modulation in the digestibility of nutrients caused by these beneficial microorganisms, improving intestinal digestion (Upadhaya *et al.*, 2015). Animals feed with D4 showed a good yield for productive variables, increasing DWG and decreasing FC, which is consistent with Li *et al.* (2012), who reported that the addition of an essential oil (100 and 150 ppm) significantly increased DWG and FC in weaned pigs, given by stimulation of digestive enzyme secretion and stabilization of the gut microbiota ecosystem (O ' Bryan *et al.*, 2015). The animals of D5, showed better performance compared to the pigs of CD and D2. It has been shown that combinations of organic acids have had positive effects on the digestibility of nutrients, as well as the growth performance of pigs (Long *et al.*, 2018).
The inclusion of various antimicrobial antibiotics, especially *B. subtilis* (D3) in pig diets in postweaning period, improve production parameters, increasing DWG, feed intake and final weight, and in turn decreasing FC. In addition, the alternatives used in this work do not represent a risk of resistance by pathogenic intestinal microorganisms, nor of transfer of resistance to bacteria in humans.

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Expression of intestinal barrier proteins in pigs added with natural antimicrobials and weaned at 28 days of age

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Introduction

Weaning is the most critical period during the life of the pig, because the digestive system must be adapted to a solid diet at the time of weaning (Ospina *et al.*, 2011). Intestinal epithelial cells are joined through tight junctions (TJ), which regulate the barrier's permeability and intestinal integrity. According to Du *et al.*, (2018), the function of the intestinal barrier is closely related to the immune response during weaning, affecting the expression of TJ proteins (Claudins-Cl, Ocludins-Ol and Zonula ocludens-ZO) essential for Maintain intestinal health. In addition, the use of Antibiotics (GPA) in animal production has been implemented to prevent the appearance of intestinal disorders, infections and diarrhea (Robinson *et al.*, 2015). However, natural products have been investigated to replace or substitute GPAs, including probiotics (Herrera *et al.*, 2015), acidifiers (Mocherla and Ramana, 2015) and plant extracts (Madrid *et al.*, 2018). The objective of this work was to quantify the expression of intestinal barrier proteins (TJ) in pigs added with natural antimicrobials and weaned at 28 days of age.

Materials and Methods

75 piglets (females and males) were used, weaned at 28 days of age with a weight of approx. 7.8 ± 0.4 kg. The animals were housed in groups of 5 animals and each group randomized to one of 5 diets: Control Diet (CD): Balanced food without antimicrobial; Diet 2 (D2): CD with antibiotic (Zinc Bacitracin) (350 ppm); Diet 3 (D3): CD with *Bacillus subtilis* pb6 (PTA 6737) (50 ppm of food. 10^8 CFU); Diet 4 (D4): CD with oregano essential oil (Lippia origanoides) (AEO) (150 ppm); Diet 5 (D5): CD with organic acid (AO), mixture of acids: citric, fumaric, benzoic, sorbic, ascorbic, lactic and sodium butyrate (4ppm). Humanitarian euthanasia was performed on 35 piglets as follows: day 1 (weaning day), 5 piglets, as a reference group to verify the general state of health. On the 15 and 30 postweaning, 3 piglets of each treatment were sacrificed (5 diets * 2 samples). 5 cm of duodenum were taken and RNA from tissues collected and conserved in liquid nitrogen was extracted using the ULTRACLEANT MT issue & Cells RNA Isolation kit (MO MO BIO Laboratories Inc Kit, San Diego, CA, USA). The expression of the proteins of interest was measured using the QuantiNova SYBR Green RT-PCR Kit (QIAGEN).

Results

The control diet (CD) animals had the lowest values for barrier protein expression ($P < 0.05$). On the other hand, piglets belonging to D3 (probiotic) groups had the highest expression ($P < 0.05$) of Cl-1, Cl-4, Ol and ZO-1 compared to pigs in group D2 (antibiotic).

Discussion and Conclusions

Epithelial barrier dysfunction is one of the main causes of intestinal problems in animals (Hsieh *et al.*, 2015); therefore, the expression of TJ proteins is essential to maintain the intestinal health of piglets during weaning (Robinson *et al.*, 2015). According to the results of this work, pigs added with probiotics presented the greatest increase in gene expression of TJ proteins during weaning, since according to Ciro *et al.*, (2016), these products can help in maturation and development of the barrier, positively modulating intestinal integrity. All of the above would allow piglets to be better adapted to the changes that occur during the weaning phase, such as the separation of the mother, the change in the presentation of the food and the low food intake (Jayaraman and Nyachoti, 2017) .

The inclusion of antimicrobials, different antibiotics, in the diet of weaned pigs, especially *B. subtilis*, increases the expression of barrier proteins, which could decrease epithelial barrier dysfunction, intestinal permeability and translocation of luminal antigens , which in turn could improve pig production.

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Application of Hemicell HT™ – a β-mannanase enzyme – restores post-weaned piglet performance in the presence of challenging protein sources

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Introduction

β-Mannans are strongly anti-nutritive polysaccharide fibres found in most vegetable feed ingredients (1).
They belong to the hemicellulose fraction and have a backbone composed entirely of mannose, as in mannans and galactomannans, or of mannose and glucose, as in glucomannans and galacto-glucomannans (2,3). The estimated content of soluble β-mannans in common nursery diets is only 0.15-0.35%, and *in vitro* studies have demonstrated that as little as 0.05% soluble β-mannan in feed can elicit a strong innate immune response (4). This innate response is often referred to as a feed induced immune response or FIIR, which suppresses growth to protect the liver and reserve energy and nutrients for high priority immune functions. Hemicell HT (Elanco) is a β-mannanase enzyme for animal feeds that breaks down β-mannans and thereby prevents economic losses from the wasteful immune response to β-mannans.

Materials and methods

A seven weeks feeding trial was conducted on a commercial sow farm with DanBred x Belgian Pietrain piglets weaned at 21 days of age. The trial was performed in 2019 with 896 pigs; all vaccinated with Coliptotec™, in two rotations of 448 piglets in 32 pens of 14 pigs. Standard three-phase control diets were compared to similar isonutritive diets with 300 g/tonne of Hemicell HT Dry except for following changes: Phase 1 (weeks 1-2): 1.14% potato protein concentrate and 1.00% Forcital (extruded soya product), was replaced with soybean meal. Phase 2 (weeks 3-4): 0.46% potato protein concentrate and 0.68% Forcital was replaced with soybean meal. Phase 3 (weeks 5-7): Hemicell HT was formulated to replace 63 kcal/kg NE. Standard production and health data were collected, including bodyweight, feed intake, faecal scores (scale 0-2), uniformity/appearance (score 0-5), medications and mortality with feeder (two pens) as experimental unit for feed intake and feed conversion and pen for all other observations. The data were analysed for each feeding period separately and overall. Analysis was performed using JMP 14.0 statistical program.

Results

In phase 1, the pigs on control feed gained 8 g/day more than on Hemicell HT ($P < 0.05$) and both feed intake and FCR were similar ($P > 0.22$). No performance differences were observed in phase two and three, and overall, very similar performance of Control and Hemicell HT was observed on all performance parameters ($P > 0.34$), except mortality, which was 1.34 %-points lower on Hemicell HT ($P = 0.067$). This correlated with a significant reduction in the use of antibiotic treatments, which was twice as high on Control as on Hemicell HT, 20.5% *vs.* 9.2% ($P < 0.01$). Diarrhoea accounted for 52-53% of all treatments and occurred from 5-13 days post-weaning.

Table 1. Overall results of comparative trial using Hemicell HT combined with challenging protein sources

	Control	Hemicell HT	*P*
Initial BW, kg	4.947	4.958	0.907
Final BW, kg	21.621	21.428	0.445
Feed intake, kg/d	0.533	0.535	0.937
Daily Gain, kg	0.340	0.336	0.347
FCR, kg/kg	1.584	1.604	0.779
Mortality, %	1.79	0.45	0.067

Conclusions and Discussion

The trial demonstrated that the use of Hemicell HT to degrade β-mannans in nursery diets made it possible to reduce the feed cost by replacing part of the expensive proteins and reduce dietary energy by 63 kcal/kg NE in phase 3 without reducing nursery pig performance or health.

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Key words: β-mannanase, β-mannan, enzyme, nursery pigs.

Yeast extract acceptability by newly weaned piglets

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Introduction

The use of ingredients that improved the palatability of the feed has been studied as an alternative to increasing feed intake and performance of weaned piglets (2,3,5). In challenging periods as post-weaning, the animals through the nutritional transition associated with the loss of contact with mother and adaptation to a new environment can present a decrease in the feed intake, which can also be influenced by the palatability of the feed (4). Ingredients derived from yeast are rich in amino acids, peptides and nucleotides, in addition, are considered of excellent palatability and digestibility (1). This study aimed to analyze if the inclusion of a yeast extract (YE) can influence the feed intake in weaned piglets.

Materials and Methods

The experiment was conducted at a commercial swine farm. For this, 250 newly weaned piglets (hybrid Topigs x Agroceres) with 24 ±4 days of age, were distributed in 5 pens (considered as experimental units), with 50 animals. The trial period was 3 calendar days, during 3 first weeks post-weaning, observations were made twice a day (8 am and 2 pm). The YE (Flavor's UP® from ICC Brazil Company) was included in the concentration of 0, 2.5%, 5% and 10% into portions of 400 g of feed. To evaluate the different acceptability, the treatments were provided in individual feeders, being one in each pen. For the feeding preference evaluation, treatments were supplied simultaneously at the pen. When the first treatments were fully consumed, all the feeders were taken out and leftover feed were weighted. At each observation the pens and positions of feeders were exchanged. The data were analyzed by Kruskal Wallis test and the treatments compared by Dunn test, performed using the software SAS Statistical Analysis System, version 9.4 (SAS Inc., Cary, NC, USA).

Results

The averages of feed intake in the acceptance test are showed in Table 1. Statistical significant differences were not observed for acceptance. The results of the analysis of the feeding preference, averages of feed leftovers are shown in Table 2. A significant difference was found between the concentration of 10%, compared to others that do not differ.

Conclusions and Discussion

The inclusion of different levels of the yet was also accepted and consumed. However, when measured the animals feeding preference the inclusion of 10% was statistically higher mean, compare with the other available treatments.

Table 1. Feed intake of newly piglets receiving different levels of yeast extract (acceptability).

YEAST EXTRACT INCLUSION (%)	FEED INTAKE AVERAGE (g)
0	0.256
2.5	0.271
5	0.273
10	0.293
p-value	0.749

Table 2. Feed leftovers of newly piglets receiving different levels of yeast extract (feeding preference).

YEAST EXTRACT INCLUSION (%)	FEED INTAKE AVERAGE (G)
0	0.155[a]
2.5	0.190[a]
5	0.165[a]
10	0.013[b]
p-value	<.0001

[(a,b)]Superscripts indicate statistically significant differences within the main effect (*p <0,001*).

The data suggested that the inclusion of 10% YE was preferred by the piglets, stimulating feed intake. This statement is supported by the fact that both treatments were equally consumed when supplied separately. This indicates that all inclusions are accepted, but the inclusion of 10% YE is more attractive.

Acknowledgments

ICC Brazil, São Paulo, Brazil.

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The influence of different sucrose solution concentrations on time budget of exploratory and social behaviors in nursery pigs

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Introduction

Pigs innately detect and prefer sweet compounds **[1],** increasing the pleasure they perceive as the inclusion of carbohydrates such as sucrose increases **[2].** This could increase motivation and competition for obtaining such resources, generating agonist behaviors in animals that receive sweet additives. In addition, animals could also modify the time budget that they allocate to other behaviors during the consumption period of palatable resources. The objective of the present experiment was to quantify and relate the time budget of exploratory, feeding and social behaviors to different concentrations of sucrose solutions delivered to nursery pigs.

Materials and Methods

A total of 24 nursery pigs were allocated in 12 pens (2 pigs/pen) and daily exposed (10 min), during 7 consecutive days, to different sucrose solutions (0.5; 1; 2; 4; 8; 16 and 32%). Animals were video-recorded in order to determine the time budget they spent during the consumption tests on exploratory (locomotion, environment exploration, feeder exploration, feeder rooting), and social behaviors (near the feeder, away from the feeder, agonistic near the feeder and agonistic away from the feeder). The effect of sucrose inclusion on the measured behaviors was analyzed (SAS®).

Results

In relation to exploratory behaviors (Figure 1a), sucrose concentration affected locomotion (P=0.029), observing the highest values with the lowest concentrations. Sucrose concentration also had an effect on environment exploration (P<0.01) observing the highest values at intermatiate concentrations. Finally, feeder exploration (P=0.012) and feeder rooting (P<0.001) were affected by sucrose concentration, observing the higher values at the highest and lower concentrations respectively.

In relation to social behaviors (Figure 1b), sucrose concentration had an effect on social behaviors near (P=0.001) and away from the feeder (P<0.001), observing at the higher sucrose concentrations fewer social interactions near the feeder but higher social interactions away from it. Agonistic behaviors near the feeder or away from the feeder were also affected by sucrose concentrations (P = 0.004 and P = 0.005 respectively), where at the higher sucrose concentrations pigs presented more agonistic behaviors near the feeder but less agonistic behaviors away from it.

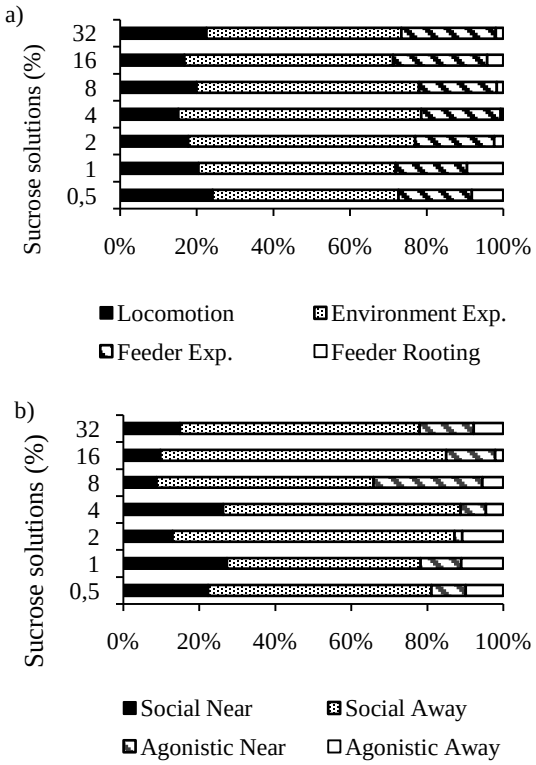

Figure 1. Time budget of exploratory (a) and social (b) behaviors (expressed as a percentage of behaviors analyzed) in nursery pigs' pairs that were exposed to different sucrose concentrations in water solutions during 10 min.

Conclusions and Discussion

Pigs presented more locomotion and feeder rooting when the reward was low probably because of the loss of interest in the solution, and presented more exploration (environment and feeder) when the reward increased probably due to high motivation to seek for such resources. Pigs presented more social interactions away from the feeder and agonistic bahaviors near the feeder when the reward was high, probably because they concentrate agonistic behaviors near the nutrient source. The perceive reward (palatability due to energy concentration) may modify time budget for exploratory and social behaviors in pigs.

Acknowledgments

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Sucrose inclusion in gestating and lactating diets of sows decreased the consumption patterns of pigs for sweet but not umami solutions after weaning

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Introduction

Pigs show innate gustatory preferences for simple carbohydrates (sweet taste) and amino acids (umami taste). However, flavor experiences during gestation and lactation could modify the feeding behavior of pigs, e.g. when an umami taste compound (monosodium glutamate, MSG) is included into maternal diets (1). The aim of this study was to evaluate the effect of sucrose inclusion in gestating and lactating diets of sows on consumption patterns of their progeny for sucrose and MSG solutions.

Materials and Methods

Twenty-two sows (Landrace × Large White) and 208 male and female piglets from their litters were assigned to 2 experimental groups offered standard commercial gestation and lactation feeding programs without (control group) or with (sucrose group) the inclusion of 50 g/kg of sucrose. Animals born from these sows were distributed into 4 pens of 26 animals of the same age, for each experimental group at weaning (21 days). Piglets were then offered in pairs a single drinker containing four different concentrations of sucrose (1, 6, 12 and 18 mM) and MSG (1, 3, 9 and 27 mM) solutions for two minutes. During that exposure, animals were recorded by video-cameras placed in the front of each pen, in order to assess the consumption patterns (consumption time/number of approaches) of the solutions as a potential measure of their palatability (2). Data was analyzed with ANOVA by using the GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA).

Results

Consumption patterns of pigs born from control or sucrose-fed sows during the 2-minute exposure to sucrose and MSG solutions are shown in Table 1. The pattern for sucrose solutions was lower in pigs coming from sucrose-fed sows in comparison with that of animals coming from control sows at 1 and 6 mM. A tendency to a lower consumption pattern value was also observed at 12 mM sucrose in pigs whose mothers were fed 50 g/kg of sucrose during gestation and lactation, while no differences were observed at 18 mM. In contrast, no differences in consumption patterns of MSG solutions were observed among pigs born from control or sucrose-fed sows. These latter animals even showed statistical tendencies to higher values at 3 and 9 mM MSG.

Table 1. Consumption patterns of sucrose and MSG solutions in post-weaning pigs born from control or sucrose-fed sows.

SOLUTION	GROUP[1]		SEM	*p*-Value
	Control	Sucrose		
Sucrose (mM)				
1	5.1	3.8	0.35	0.014
6	6.3	4.0	0.39	<0.001
12	6.2	5.2	0.35	0.065
18	6.4	5.4	0.54	0.179
MSG (mM)				
1	5.7	6.0	0.46	0.616
3	7.0	8.5	0.59	0.073
9	5.6	7.0	0.58	0.090
27	7.0	7.7	0.75	0.504

[1]From ANOVA analysis, including the effect of experimental group [control (pigs born from sows fed standard gestating and lactating diets) or sucrose (pigs born from sows fed gestating and lactating diets included with 50 g/kg of sucrose)]. Treatment n = 13. Mean values are least-squares means with a significance level of $p < 0.05$.

Conclusions and Discussion

It was observed that the inclusion of 50 g/kg of sucrose in gestating and lactating diets of sows decreased the consumption patterns of pigs for low-concentration sucrose solutions (1 and 6 mM). However, no effect was observed at the highest sucrose concentration tested (18 mM), or any of the MSG solutions evaluated. Previous studies in nursery pigs have associated consumption patterns to palatability, or the hedonic perception of animals with solutions/feeds (2). Thus, these results may indicate that the hedonic perception of sweet compounds in the post-weaning period can be manipulated by a higher pre and postnatal sweet inclusion in mothers' diet, and that this effect might be taste-specific as no differences were detected in the hedonism of umami compounds.

Acknowledgments

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Sucrose inclusion in gestating and lactating diets of sows modified the locomotion and affiliative behaviors of post-weaning pigs for sweet solutions

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Introduction

It has been shown in a recent study that a perinatal exposure to a high-fat high-sugar diet influences the behavioral development of pigs after weaning, as observed by increased interactions with pen mates, decreased aggressiveness and/or increased walking time in animals exposed to the diet high in saturated fats and refined sugars, as compared to controls (1). While the diet used in that study contained 12% saturated fat, 18.5% sucrose and 1% cholesterol, no information exists about a likely programing effect of pure sucrose inclusion on the behavior of pigs. Therefore, the aim of this work was to evaluate the effect of the inclusion of 5% sucrose in gestating and lactating sows' diets on their progeny behavior when offered sucrose solutions after weaning.

Materials and Methods

Twenty-two sows (Landrace × Large White) and 208 male and female piglets from their litters were assigned to 2 experimental groups offered standard commercial gestation and lactation feeding programs without (control group) or with (sucrose group) the inclusion of 5% of sucrose. Animals born from these sows were distributed into 4 pens of 26 animals of the same age, for each experimental group at weaning (21 days). Piglets were then offered in pairs a single drinker containing four different sucrose solutions concentrations (1, 6, 12 and 18 mM) for two minutes. During that exposure, animals were recorded by video-cameras placed in the front of each pen, in order to assess their exploratory, alimentary and social behaviors. Videos were subsequently analyzed with The Observer XT (Noldus, Wageningen, the Netherlands) by using the pair of pigs as experimental unit. Data was analyzed with ANOVA through the GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA), considering the effects of experimental group, concentration tested and their interaction as main factors.

Results

In comparison with animals coming from control sows, pigs born from sucrose-fed sows showed a reduced walking time within pens (Table 1). On the other hand, pigs whose mothers were fed 5% sucrose during gestation and lactation showed a higher number of interactions with the drinkers and higher affiliative behaviors with pen mates. No differences were observed in all the other parameters analyzed in this study due to the group effect. The time standing in the pen was affected by the group*concentration interaction, with sucrose pigs who tested 6 mM sucrose spending more time standing than control animals at 18 mM sucrose.

Table 1. Behavioral parameters of pigs born from control or 5% sucrose-fed sows when offered sucrose solutions after weaning[1].

BEHAVIOR	GROUP		SEM	*p*-VALUE		
	Control	Sucrose		G	C	G*C
Exploring	4.56	3.91	0.95	0.628	0.102	0.427
Walking	18.35	14.80	1.30	0.057	0.037	0.638
Standing	14.55	13.94	1.49	0.772	0.001	0.044
Ingesting	76.92	72.24	3.01	0.275	0.068	0.172
Interactions	7.48	8.37	0.28	0.030	0.857	0.070
Approaches	1.91	2.06	0.10	0.311	0.002	0.329
Affiliative	0.03	0.20	0.05	0.014	0.779	0.366
Agonistic	0.20	0.17	0.06	0.720	0.623	0.701
Resting	0.00	0.75	0.39	0.171	0.553	0.553

[1]From ANOVA analysis, including the effects of experimental group [control (pigs born from sows fed standard gestating and lactating diets) or sucrose (pigs born from sows fed gestating and lactating diets included with 5% sucrose); G], concentration (1, 6, 12 and 18 mM; C) and their interaction (G*C). n = 13. Mean values are least-squares means with a significance level of $p < 0.05$. Only means due to group effect are showed.

Conclusions and Discussion

Results of this study confirm that the inclusion of a taste-active compound such as pure sucrose in prenatal and postnatal diets influences some behavioral characteristics of pigs during nursery. In contrast to the work of Clouard et al. (1), we observed that pigs born from sucrose-fed sows reduced their time walking during solutions exposure. It is important to note that these authors probed a diet with a much higher dose of sucrose (18.5%) and included with saturated fats than the one used here, with the aim of reflecting the patterns of human western diets. Nonetheless, similar to that study, we also observed with the inclusion of just 5% sucrose a decreased aggressiveness of animals after weaning, as reflected by higher affiliative behaviors with conspecifics. The physiological pathways by which sucrose derivatives influence the progeny warrant further research.

Acknowledgments

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Short-chain fatty acids profile in cecum and colon of piglets fed diets containing dry, reconstituted or ensiled sorghum grains

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Introduction

While most of the starch is digested in the small intestine, a fraction of it can escape digestion there and be fermented in the hindgut by the microbiota. The fermentation of resistant starch and other indigestible carbohydrates leads to the production of short-chain fatty acids (SCFA), which can be absorbed providing energy to the animal (1). The nature of starch fermented can also modify the profile of SCFA produced in the large intestine. The increase in the content of resistant starch in the diet of piglets was associated with an increase in the molar proportion of butyrate in the hindgut, which was related to the larger size of the colon (2). The objective of this study was to determine whether the inclusion of dried or fermented sorghum grains in diets for weaned piglets is related to modifications in the in the production profile of SCFA in the large intestine.

Materials and Methods

Twenty-seven castrated crossbreed male piglets (Landrace x Large White; initial BW 8.60 ± 1.88 kg) were used in the study. Piglets were individually allocated in metabolic crates (0.9 × 1.2 m), blocked by weight and assigned to one of three treatments. Treatments consisted of diets based on dry sorghum grain (DRY), reconstituted sorghum grain (REC), and ensiled high moisture sorghum grain (ENS). The grains came from a single sorghum crop divided in random plots, and the experimental design was a randomized block assay with three treatments and nine replicates. At the end of the trial (day 24), piglets were unsensitized by electric stunning and bleeding euthanized, the gastrointestinal tract (GIT) was immediately removed, and digesta from the cecum and ascending colon was sampled and stored at -20°C until SCFA determination.

Concentrations of SCFA (acetate, propionate, isobutyrate, butyrate, isovalerate and valerate) were analysed according to Adams et al. (3). Proportions of acetate, propionate and butyrate were calculated in relation to the total SCFA, and branched-chain fatty acids (BCFA) were calculated as isobutyrate + isovalerate + valerate. Data was analysed using the MIXED procedure of SAS (SAS 9.0V, SAS Institute Inc., Cary, NC), and the mean comparisons were made using the Tukey test. Statistical significance was considered at $P < 0.05$ and tendencies at $P < 0.10$.

Results

Total SCFA production was similar among treatments in both caecum and colon. In the caecum, the proportion of acetate was higher, and that of propionate was lower in piglets fed with ENS, while the proportion of butyrate tended to be lower in those fed with REC. In the colon, the proportion of butyrate was lower in those fed with REC.

Table 1. Total short-chain fatty acids ($SCFA_{tot}$), branched-chain fatty acids (BCFA) and molar ratios of acetate, propionate and butyrate in caecal and colonic digesta of piglets fed diets containing dry (DRY), reconstituted (REC) or ensiled (ENS) sorghum grains.

	DRY	REC	ENS	SEM[c]	P-value[d]
			Caecum		
$SCFA_{tot}$, mM	457.6	478.0	434.8	26.28	0.519
BCFA, mM	69.8^{ab}	85.4^{a}	43.7^{b}	11.17	0.044
Acet, %	46.2^{b}	50.2^{ab}	56.6^{a}	1.88	0.021
Prop, %	24.6^{a}	23.8^{a}	20.3^{b}	0.93	0.006
But, %	14.1^{x}	8.9^{y}	13.4^{xy}	1.33	0.065
			Colon		
$SCFA_{tot}$, mM	464.6	469.4	412.3	27.55	0.201
BCFA, mM	81.9	81.6	57.5	12.07	0.281
Acet, %	45.7	52.2	50.9	3.28	0.400
Prop, %	22.9	22.3	22.6	2.23	0.983
But, %	13.6^{ab}	8.7^{b}	14.5^{a}	1.24	0.033

[c] Standard error of the mean (n=9). [d] Level of treatment significance; different letters in the same row differ ($P < 0.05$) or tend to differ ($P < 0.10$).

Conclusions and Discussion

Although no differences in SCFAtot and absolute amounts of the different types of fatty acids were detected, piglets fed DRY and ENS treatments had higher molar proportions of butyrate, which could be associated with healthier fermentation profiles in the colon. Differences in the type of carbohydrate reaching the large intestine may be related to these modifications in the SCFA production profile.

Acknowledgments

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In vitro fermentation of diets for piglets containing dry, reconstituted or ensiled sorghum grains

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Introduction

In vitro fermentation models can be used to simulate the fermentative profiles of the microbiota within the intestine and its relation with the intestinal physiology and health (1). Quantitative and qualitative production of SCFA, as well as fermentation kinetics of indigestible carbohydrates, including undigested starch in the small intestine, can be evaluated through these methods (2). Treatments conducted on cereal grains can modify its digestibility, and therefore its fermentability. The aim was to characterize the fermentation kinetics and SCFA production of the faecal microbiota of piglets fed diets containing dry, reconstituted or ensiled sorghum grains using an *in vitro* fermentation system.

Materials and Methods

Diets for piglets based on dry sorghum grain (DRY), reconstituted sorghum grain (REC), and ensiled high moisture sorghum grain (ENS) were pre-digested with pepsin and pancreatin solutions. The residues were dried, placed in 100 mL bottles (n=72), and an incubation media (30 mL) and diluted fresh faeces (10 mL) were added. Faeces were obtained from piglets fed DRY, REC or ENS. Incubation was conducted in water bath at 37°C and gas production was measured in the bottles at 2, 4, 6, 8, 10, 12, 18, 24, 48, and 72 h. Asymptotic gas production (A, mL/g OM), time to reach 50% of the asymptote (C, h), maximal rate of gas production (R_{max}, mL/h), and the time of its occurrence (T_{max}, h) were determined. A second set of bottles were opened at 24 or 72 h for SCFA determination. Concentrations of SCFA (acetate, propionate, isobutyrate, butyrate, isovalerate and valerate) were analysed according to Adams et al. (3). Proportions of acetate, propionate and butyrate were calculated in relation to the total SCFA, and branched-chain fatty acids (BCFA) were calculated as isobutyrate + isovalerate + valerate. Data was analysed using the MIXED procedure of SAS (SAS 9.0V, SAS Institute Inc., Cary, NC), and the mean comparisons were made using the Tukey test. When interactions were significant, simple effects were tested using the "slice" option.

Results

The DRY treatment showed a lower *in vitro* OM digestibility, a higher gas production, but with a slower fermentation speed (higher C, lower R_{max} and higher T_{max}) than the others. The production of fatty acids was greater at 72 h of incubation (Table 2). Interactions were observed between the diet effect and the incubation time in the concentrations of SCFAtot, BCFA, acetate and propionate. The highest productions of SCFAtot, BCFA and propionate were observed at 72 h of incubation in animals that consumed DRY. Acetate production at 24 h of incubation was higher in animals that received ENS.

Table 1. *In vitro* fermentation parameters for different diets.

	Diet[d]				
	DRY	REC	ENS	SEM[e]	P-value[f]
OMIVD[g]	0.47[b]	0.59[a]	0.58[a]	0.013	<0.01
A, mL/g OM	289[a]	249[b]	211[c]	13.9	<0.01
C, h	12.4[a]	8.2[b]	6.8[b]	1.61	<0.01
R_{max}, mL/h	14.0[b]	18.7[a]	18.3[a]	0.98	<0.01
T_{max}, h	3.42[a]	2.00[b]	1.54[b]	0.38	<0.01

[d]Diets containing dry (DRY), reconstituted (REC) or ensiled (ENS) sorghum grains [e]Standard error of the mean (n=72). [f]Different letters in the same row differ (P < 0.05). [g]*In vitro* digestion of OM.

Table 2. *In vitro* total short-chain fatty acids ($SCFA_{tot}$), branched-chain fatty acids (BCFA) and acetate, propionate and butyrate production (mM/g OM) for different diets.

		Diet[c]				P-values[e]		
	h	DRY	REC	ENS	SEM[d]	*D*	*t*	*D*×*t*
$SCFA_{tot}$	24	195	196	222	12.9	0.13	<0.01	<0.01
	72	269[a]	237[b]	235[b]				
BCFA	24	16.2	16.3	16.3	4.6	0.21	<0.01	0.03
	72	29.9[a]	19.8[b]	18.0[b]				
Acet	24	104[b]	112[b]	130[a]	8.3	0.31	<0.01	<0.01
	72	148	140	139				
Prop	24	71.4	70.1	69.3	5.6	0.14	<0.01	<0.01
	72	90.9[a]	78.1[b]	74.8[b]				
Buty	24	22.9	16.7	22.9	4.3	0.15	<0.01	0.10
	72	32.6	21.1	24.4				

[c]Diets containing dry (DRY), reconstituted (REC) or ensiled (ENS) sorghum grains. [d]Standard error of the mean (n=72). [e]Effects diet (*D*), time (*t*) and their interaction; different letters in the same row differ (P < 0.05).

Conclusions and Discussion

Diets containing dry grains were associated with lower digestibility, and greater but slower fermentation than REC and ENS diets, suggesting a strong effect of sorghum grain treatment on nutritional parameters. The highest fermentation observed in DRY is consistent with a higher production of SCFA and BCFA. The higher production of acetic acid in ENS indicates a higher proportion of complexes CH reaching the large intestine.

Acknowledgments

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Reconstituted or ensiled sorghum grain: effects on *in vitro* fermentation and short-chain fatty acid profiles in pigs

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Introduction

Sorghum grain is used in animal nutrition as a source of energy; however, due to its low digestibility it is necessary to apply treatments on it (1). The early harvest and the preservation of the grain under anaerobic conditions improves sorghum digestion in pigs (2,3). Starch that resists digestion in the small intestine can be fermented in the hindgut, and therefore, treatments conducted on the grains may have implications in large intestine fermentation too. We studied the *in vitro* fermentation profile of sorghum grains ensiled or reconstituted to be used in pig feeding.

Materials and Methods

A sorghum crop was divided into 9 plots and subjected to three treatments following a randomized block design: grains harvested at 40% moisture and ensiled (EG), grain harvested dry and reconstituted by the addition of water up to 40% moisture (RG), and grain harvested dry (DG). EG and RG grains were grounded and anaerobically stored in silo drums for 180 d. Grains were pre-digested with pepsin and pancreatin and the residues were incubated in a 100 mL bottles. An incubation media (30 mL) containing diluted sow faeces was added. Bottles (n=44) were incubated at 37ºC, gas production was measured during 72 h, and fermentation kinetics [asymptotic gas production (A), time to reach 50% of the asymptote (C), maximal rate of gas production (R_{max}), and the time of R_{max} occurrence (T_{max})] were determined. Bottles were opened at 72 h, along with another set of bottles incubated for 24 h for analysis of short-chain fatty acids (SCFA), branched chain fatty acids (BCFA) and molar proportions of acetate, propionate and butyrate. Data was analysed using the MIXED procedure of SAS; means were compared using orthogonal contrasts for *in vitro* kinetics, or Tukey test for SCFA profiles.

Results

Dried grains produced more gas, but at a lower rate than treated grains (Table 1). The production of SCFA, BCFA and acetate proportion was greater at 72 h of incubation (Table 2). On the other hand, propionate proportion was lower at 72h. BCFA was higher in the EG treatment. Acetate ratio increased in the dried harvested grains (DG and RE), whereas propionate proportion was greater in fermented grains (RG and EG). Butyrate molar ratio was greater in DG than in RG. There was no interaction between time and treatment effects.

Table 1. *In vitro* fermentation parameters for dry (DG), reconstituted (RG) and ensiled grains (EG).

	Treatment				Contrasts (P)	
	DG	RG	EG	SEM	C1	C2
A, mL	315	270	259	9.5	<0.001	0.417
C, h	12.7	8.2	8.4	0.34	<0.001	0.632
R_{max}, mL/h	16.4	19.5	19.7	3.99	0.025	0.912
T_{max}, h	7.4	9.1	7.5	0.66	0.291	0.089

SEM: Standard error of the mean; Contrast: C1=DG *vs* treated grains; C2=RG *vs* EG.

Table 2. *In vitro* total short-chain fatty acids ($SCFA_{tot}$), branched-chain fatty acids (BCFA) production (mM/g DM), and acetate, propionate and butyrate molar ratios (%) for dry (DG), reconstituted (RG) and ensiled grains (EG).

		Treatment				P-values	
	h	DG	RG	EG	SEM	T_x	*h*
$SCFA_{tot}$	24	247	282	299	17.7	0.217	<0.01
	72	307	325	351			
BCFA	24	27.2^{b}	27.1^{b}	37.9^{a}	14.81	0.012	<0.01
	72	35.3^{b}	32.2^{b}	47.8^{a}			
Acet%	24	55.3^{a}	54.9^{a}	50.4^{b}	2.06	0.026	0.864
	72	55.3^{a}	54.8^{a}	50.2^{b}			
Prop%	24	26.8^{b}	30.7^{a}	31.6^{a}	0.97	0.006	0.008
	72	26.1^{b}	29.8^{a}	30.9^{a}			
But%	24	6.8^{a}	4.8^{b}	5.5^{ab}	0.74	0.048	0.316
	72	7.1^{a}	5.4^{b}	5.7^{ab}			

Tx: treatment effect; *h*: time effect; SEM: Standard error of the mean; different letters in the same row differ (P < 0.05).

Conclusions and Discussion

Untreated grains were fermented to a greater extent but more slowly than the treated grains. This could be explained by a greater amount of fermentable substrate reaching the large intestine, although more slowly fermentable by the microbiota. Variations in the substrate chemical composition induced by reconstitution or ensiling of the grains could also explain the differences in fermentation metabolites.

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This study was founded by the proyect ANII-FMV_1_2017_1_136157.

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Effect of attapulgite and benzoic acid addition to the diet of fattening pigs on the performance parameters

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Introduction

In the last decade, there is increased interest worldwide to limit the use of antibiotics in animal nutrition, while keeping a high level of animal health, welfare and productivity.For this reason, novel feed additives are examined in order to develop cost effective and efficient feeding plans (1).

In this research, an *in situ* experimental procedure was performed in a commercial farm in Greece to investigate the effect of the combined dietary supplementation of attapulgite and benzoic acid on fattening pigs' growth performance and feed efficiency.

Materials and Methods

In this experiment, pigs were handled in compliance with the local and EU regulation for the protection of animals used for scientific purposes (Directive 2010/63/EU).

In total, 120 crossbred (1/4 Large White × 1/4 Landrace × 1/2 Duroc) male and female fattening pigs were randomly allocated into 2 treatments with 4 replications per treatment (15 pigs per pen). Each pen (6m × 2.2m) was housed in the same building of a commercial farm. The diet of the control group was in meal form (based on maize, barley, wheat bran and soybean meal) and was formulated according to standard recommendations. The diet of the second group was further supplemented with 4 g/kg attapulgite (UltraFed®, 75% attapulgite, Geohellas S.A.) and 5 g/kgbenzoic acid (VevoVitall®, 100% benzoic acid, DSM Nutritional Products Ltd.) at the expense of maize grain. Standard husbandry conditions were used throughout the feeding period. Access to feed and drinking water was *ad libitum*. The duration of the experiment was 59 days, starting at 111 (±1) days of age and finishing in 170 (±1) days of age.

The collected data was subjected to one-way ANOVA, using the IBM SPSS Statistics v. 20.0 Statistical Package (SPSS Inc., Chigaco, IL, USA). Significance was set at 5% ($P<0.05$).

Results

Table 1 presents the effect of the dietary supplementation on the fattening pigs' performance parameters. The pigs that were fed the supplemented diet had significantly ($P\leq0.05$) higher final body weight at the 170th day of age, higher total weight gain, higher average daily weight gain, lower total feed intake and improved feed conversion ratio, compared to the pigs that consumed the control diet.

Table 1. Effect of the combined dietary supplementation of attapulgite (4 g/kg) and benzoic acid (5 g/kg) on performance parameters of fattening pigs.

Experimental Period	**Treatments (Means ± SD)**	
Days of age: 111-170	**Control**	**Supplemented**
Live-weight, kg		
Day of age: 111th	54.3±4.1	54.2±4.4
Day of age: 170th	99.5^{a}±3.6	104.5^{b}±4.3
Total Weight Gain, kg	45.2^{a}±2.5	50.3^{b}±3.2
Daily Weight Gain, kg/day	0.78^{a}±0.08	0.85^{b}±0.08
Total Feed Intake, kg	180.5 ^{b} ±8.1	169.5 ^{a} ±6.1
Feed Conversion Ratio (feed/gain)	3.99^{b}±0.21	3.37^{a}±0.19

^{a,b} Mean values with different superscripts differ significantly ($P\leq0.05$).

Conclusions and Discussion

The use of antibiotics as growth promoters has been banned in the European Union since 2006 and in an increasing number of other countries every year. A variety of different feed additives is under examination today. One such category are the clay mineral, such as attapulgite, a naturally occuring crystalline hydrated magnesium alumino-silicate (2). Another important category of feed additives are the short chain fatty acids such as benzoic acid (3). Another important consideration is that the combined use of feed additives may lead to a synergistic effect and better overall result of the swine gut function.

Based on the results of this feeding trial, the combined supplementaion of attapulgite and benzoic acid in the diet of fattening pigs could enhance their growth and improve the feed efficiency.

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Can protein levels be reduced in the post-weaning diet to match the effect of medicinal zinc?

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Introduction

Medicinal zinc prevents post-weaning diarrhoea (PWD) in weaner pigs (2,6). In EU it has been banned from 2022 and an increased post-weaning antibiotics usage is expected (1). Previous studies demonstrated a positive effect of a low protein diet on PWD (3,4). However, feeding low protein levels also decrease growth performance, why it is important to include free amino acids (5). Therefore, the objective of the present study was to determine if a low protein strategy with two different types of protein source could decrease the diarrhoea frequency and thereby the antibiotics usage, without compromising the productivity.

Materials and Methods

The trial was conducted at a research unit with a high health status, and included 1,579 DanBred pigs from weaning at 5.5-9.0 kg until around 30 kg. Pigs were fed a three-phase diet and the protein levels are presented in table 1. Group 1 received 2,500 ppm zinc in the first phase as a positive control group, whereas group 2 received the same diet without medicinal zinc as a negative control.

Table 1. Digestible protein levels in the different dietary treatment groups in the three feeding phases.

Group	1	2	3	4	5
Phase 1 6-9 kg*	19.2	19.1	17.6	17.7	15.4
Phase 2 9-15 kg*	18.9	18.9	17.4	17.6	15.1
Phase 3 15-30 kg*	19.1	19.1	19.1	19.1	19.1

*% Crude protein in the diet

Groups 3 and 5 received an expensive diet with a high percentage of soy protein concentrate and a low amount of soybean meal, whereas group 4 received a cheaper diet with a high amount of soybean meal and less protein concentrate. All groups received lysine, methionine, tryptophan, threonine and valine to compensate for the low protein diet, whereas group 5 additionally received synthetic isoleucine, leucine, phenylalanine, histidine and tyrosine to test the potential of these amino acids.
The effect of feeding strategy on diarrhoea treatments were analysed using a logistic regression model in R, followed by a Bonferroni adjusted comparison. Productivity data were analysed using a mixed effect model in R, followed by a Tukey adjusted comparison.

Results and Discussion

Preliminary results suggest that the group allocated 2,500 ppm zinc in phase one, had 30% fewer diarrhoea treated pigs at day 39 than group 2 (table 2). The expensive and cheap groups did not differ in the percentage of antibiotics treated pigs at the end of the trial, whereas the extreme low protein group had a 70% reduction in the number of treated pigs at 30 kg compared with group 2 (table 2).

Table 2. Percentage of antibiotics treated pigs at day 10, 24 and 39 post weaning.

Group	1	2	3	4	5
No. of pigs	263	525	263	264	264
Day 10*	1.1[a]	4.8[a]	8.0[a]	3.0[a]	2.6[a]
Day 24*	22.0[b]	30.4[a]	38.7[a]	27.7[a]	12.6[b]
Day 39*	38.9[b]	49.9[a]	46.8[a]	40.4[a]	15.8[b]

*Different letters (a, b) within a row indicate a significant difference ($P < 0.05$) with group 2.

The difference in ADG between the two control groups with and without 2,500 ppm zinc (401 vs. 402 g/day) was not significantly different. The ADG from 7-30 kg between the groups receiving expensive (382 g/day) and cheap (383 g/day) low protein diets and group 2 (402 g/day) were not significantly different. On the other hand, the group receiving extreme low protein had a significantly decreased ADG (311 g/day, $P < 0.05$) compared with group 2. This may be due to an overestimate of the amino acids in the raw feeds, and is revised in the remainder of the trial.

Conclusions

An extreme low protein diet can be used as an alternative to medicinal zinc to reduce PWD. Based on preliminary results it is clear that the protein source does not affect the number of antibiotics treatments or the ADG of pigs post weaning However, a 15.4% protein allocation post-weaning result in significantly fewer diarrhoea treatments from weaning to 30 kg.

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Dietary oregano essential oil and organic acids change colon´short chain fatty acid profile of piglets

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Introduction

Piglets in the post-weaning period are highly susceptible to enteric bacterial infections caused by opportunistic pathogens (6). Because of concerns about the development of antibiotic-resistant strains, other alternative additives are used in the diet of piglets, such as organic acids (8) or essential oils (7). The phytogenic compounds, mainly essential oils or short chain fatty acids (SCFA) may specifically enhance activities of digestive enzymes and nutrient absorption (1,5,11), changing the piglet gut profile of SCFA. These SCFA are the main energy source to epithelial cells (butyric), important for gluconeogenesis (propionic) or lipogenesis stimulation (acetic) (1). Thus, the objective of this work was to evaluate the potential of a blend of organic acids and oregano essential oil as additives on gut short chain fatty acids profile, compared to antimicrobial treatment.

Materials and Methods

The experiment was carried out at the Swine Research Unit of School of Veterinary Medicine and Animal Science, UNESP, Campus Botucatu (Ethics Committee on the Use of Animals´ protocol 0913/2019). Forty crossbred weaned piglets with initial body weight of 5.62 ± 0.31kg (21 day of age), were distributed in a randomized complete block design, with five treatments, eight replications and the animal as experimental unit. Animals were given *ad libitum* access to a three-phases program (2) being d0-11 = pre-starter I; d12-28 = pre-starter II and d28-34 = starter, with five treatments: T_1 = basal diet (BD) without additives (control diet); T_2 = BD with 120 ppm of Halquinol (all phases); T_3 = BD with 1.0kg/ton (pre-starter I) and 0.5kg/ton (pre-starter II and starter) of oregano essential oil (*Origanum vulgare*); T_4 = BD with 3.0kg/ton (pre-starter I and II) and 1.5kg/ton (starter) of organic acids (blend of formic, propionic and butyric); T_5 = BD with 0.5kg/ton of oregano essential oil (all phases) + 1.5kg/ton of organic acids (formic, propionic and butyric) in all phases. On the 37th day of experiment, the animals were stunned by eletronarcosis and slaughtered. Samples of 10g of proximal colon digesta were collected (4) for analysis of SCFA (acetic, propionic and butyric). The data were analyzed using PROC GLM procedure (10) and the means compared by Tukey test.

Results and discussion

There is no effect of treatments in acetic, propionic and total SCFA concentration in piglet´s proximal colon (Table 1). The animals fed combination of oregano essential oil (EO) and organic acid blend (OA) had lower (P=0.0323) butyric acid concentration compared to control diet, but the treatments EO and AO individually were effective to maintain the same concentration of this acid compared to antibiotic treatment. Butyric acid provides energy to intestinal cells, support beneficial microorganisms and controls pathogens (3), which could be important in minimizing post-weaning diarrhoea. Although not significant, the animals fed EO, OA or EO+OA had higher (trend, P=0.0752) propionic acid concentration than animals fed antibiotics. These data suggests that these additives can promote changes in piglet´s colon fermentation profile, creating a more acidic environment.

Table 1. Short chain fatty acid concentration in piglet´s proximal colon at 58d of age (mMol/L)[1,2]

	Acetic	Propionic	Butyric	Total
Control diet	71.198	36.786	21.008[a]	134.898
Antibiotic	70.536	30.898	19.973[ab]	127.156
EO	75.886	36.676	19.123[ab]	137.760
OA	75.498	38.059	18.485[ab]	138.808
EO+OA	70.608	36.343	14.863[b]	127.734
CV	12.263	14.299	20.198	9.407
P value	0.5754	0.0752	0.0323	0.2647

[1]Within a column, means followed by different letter differ by Tukey test (P<0.05); [2]Antibiotic (120ppm of Halquinol); EO (oregano essential oil); OA (blend of organic acids – formic, propionic and butyric); Total = total short chain fatty acid concentration; CV (Coefficient of variation).

Conclusion

The inclusion of EO or OA individually or the combination of them may lead to the same colon acids concentration profile compared to antibiotic growth promoter, recognizing as promising alternatives to antibiotics in feeds.

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Cecal *E.coli* counts changes in piglets fed essential oil and organic acids blend

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Introduction

Due to the developing antibiotic resistance, the use of antibiotics as growth promoter has been banned in animal feed. Then, alternatives to antibiotics have been tested in swine diets as organic acids (10) and essential oils (7). The use of organic acids can modulate intestinal microbiota and enhance growth performance (5). Essential oils, such as carvacrol and thymol, have anti-microbial activity (11), because their lipophilic structure can easily get into the bacterial membrane (4). Thus, the objective of this work was to evaluate the potential of a blend of organic acids and oregano essential oil as additives on intestinal microbiota, as alternatives to antimicrobials.

Materials and Methods

The experiment was carried out at the Swine Research Unit of School of Veterinary Medicine and Animal Science, UNESP, Campus Botucatu (Ethics Committee on the Use of Animals´ protocol 0913/2019). Forty crossbred weaned piglets with initial body weight of 5.62 ± 0.31kg (21 day of age), were distributed in a randomized complete block design, with five treatments, eight replications and the animal as experimental unit. Animals were given *ad libitum* access to a three-phases program (d0-11= pre-starter I; d12-28 = pre-starter II and d28-34 = starter), formulated according to literature (6), being: T_1 = basal diet (BD) without additives (control diet); T_2 = BD with 120 ppm of Halquinol (all phases); T_3 = BD with 1.0kg/ton (pre-starter I) and 0.5kg/ton (pre-starter II and starter) of oregano essential oil (*Origanum vulgare*); T_4 = BD with 3.0kg/ton (pre-starter I and II) and 1.5kg/ton (starter) of organic acids (blend of formic, propionic and butyric); T_5 = BD with 0.5kg/ton of oregano essential oil (all phases) + 1.5kg/ton of organic acids (formic, propionic and butyric) in all phases. On the 37th day of experiment, the animals were stunned by eletronarcosis and slaughtered. Samples of 25g of proximal cecum digesta were collected for analysis *Lactobacillus* and *Clostridium* (1) and *Escherichia coli* (*E.coli*) using Dish 3M™ Petrifil™ according to manufacturer.

Results and Discussion

There is no effect of treatments in total coliforms, *Clostridium* and *Lactobacillus* counts in cecal digesta of piglets (Table 1). This result is according to others researches that failed to demonstrate the effect of organic acids (OA) or essential oil (EO) on gut microbiota (12,8). However, animals fed EO individually or the combination of EO+OA had lower (P=0.0082) *E.coli* counts compared to antibiotic treatment. Organic acids can change intestinal environment providing a non-appropriate condition for pathogenic bacteria (3) and essential oil, such as oregano, have anti-microbial activity (2), as demonstrated in our findings.

Table 1. Microbial population in piglet´s cecal digesta at 58d of age (log 10 UFC/g)[1,2]

	Total coliforms	*E.coli*	*Clostridium*	*Lactobacillus*
Control diet	7.05	6.82^{ab}	1.82	5.28
Antibiotic	7.39	7.15^{a}	1.80	5.69
EO	7.06	6.47^{b}	1.86	5.54
OA	7.14	6.90^{ab}	2.04	5.65
EO+OA	6.95	6.57^{b}	0.67	4.88
CV	4.09	5.43	76.29	11.59
P value	0.0672	0.0082	0.2195	0.0514

[1]Within a column, means followed by different letter differ by Tukey test (P<0.05); [2]Antibiotic (120ppm of Halquinol); EO (oregano essential oil); OA (blend of organic acids – formic, propionic and butyric); CV (Coefficient of variation).

Conclusion

The inclusion of EO or the combination of EO+OA can be used to replace antimicrobial growth promoter based on the inhibitory effect on counting of *E.coli*, serving as an alternative to in-feed antibiotics for piglets.

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Increased dietary TSAA through OH-Methionine in late gestating, lactating sows and weaning diet improve piglet resistance to inflammatory challenge.

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Introduction

For piglets, birth and weaning represent a major challenge to the developing immune system (2). For this, special attention should be paid to the nutritional requirement during gestation and lactation of sow. Although lysine (Lys) is the first limiting amino acid in pigs, supplementation of methionine (Met) in the sow diet can regulate milk synthesis and quality (4). Two main sources of synthetic Met are available for animal production, DL-methionine (DL-Met) and DL-2-hydroxy-4-methylthio butanoic acid (OH-Met), the later an organic acid with possible benefits in pig nutrition over DL-Met. Therefore, this study was done to check the effects of Met supplementation, as either DL-Met or OH-Met, on sows and on piglets challenged by LPS in the post weaning period.

Materials and Methods

Ten cross-bred primiparous sows per treatment were fed from d 85 of gestation to d 21 post-partum in a randomized block design with one of 3 diets: control - C (corn-soybean-DL-Met based diet meeting NRC (3) nutritional requirements), DL-Met (C + 25% DL-Met), and OH-Met (C+ 25% OH-Met). After parturition, colostrum samples were analyzed as well as milk at 14 d. At d 21, piglets were weaned and fed according to the dietary sow treatments until d 63. At d 35, 20 male piglets/ treatment were selected according to their body weight and were divided into 6 groups for a 3 × 2 factorial design trial that included 3 dietary treatments (C, DL-Met and OH-Met) and immunological challenge by intraperitoneal injection [LPS (100 µg/kg BW, E. coli 0111: B4, Sigma)] or no challenge (Saline). Piglets performance was assessed from challenge to d 63, as well plasma biochemistry. Data were analyzed by ANOVA and means compared by Tukey test (P<0.05) (XLSTAT).

Results

Colostrum from sows fed higher total sulphur aminoacids (TSSA) diets had higher level of nonfat solids (NFS), Taurine (Tau) and 3-methyl histidine (3mHys) (Table 1). In addition, the colostrum Met, Lys and Cys levels were higher with use of OH-Met than with C or DL-Met treatments. LPS challenge impaired piglet performance and increased tumor necrosis factor-a (TNF-a) and aspartate aminotransferase (AST) plasma levels (Table 2). In non-challenge group increased TSAA level did not affect performance or blood parameters compared to control. However, in challenged group, piglets receiving higher dietary TSAA level as OH-Met, showed higher body weight than control. Four hours after LPS challenge, TNF-a level from piglets fed DL-Met or OH-Met supplementation was lower than in piglets fed Control diet. OH-Met supplementation decreased AST level 24 hours after LPS challenge at similar level than non-challenge group. Increased TSAA with DL-Met was not different from control piglets for AST after 24 hours LPS challenge.

Table 1. Colostrum contents

	NFS, %	Met, µmol/l	Lys, µmol/l	Cys, µmol/l	Tau, µmol/l	3mHys, µmol/l
C	16.9a	9.8c	235c	8.8c	1465b	1.8a
DL-Met	19.8b	36.0b	412b	13.4b	2157a	0.2b
OH-Met	21.1b	54.3a	525a	22.7a	2023a	0.3b
P value	0.049	<0.001	<0.001	<0.001	<0.001	<0.001

Table 2.Performance and plasma biochemistry

Diet	LPS	DWG, kg	DFI, kg	TNF-α, ng/l	AST, UL
C	No	0.366c	0.621b	268a	90.1ab
DL-Met	No	0.375c	0.631b	291a	93.9ab
OH-Met	No	0.375c	0.634b	287a	85.7ab
C	Yes	0.233a	0.491a	2416c	174c
DL-Met	Yes	0.265ab	0.493a	1869b	148bc
OH-Met	Yes	0.281b	0.510a	1752b	80.9a
P value	LPS	<0.001	<0.001	<0.001	0.001
	Diet	0.037	0.625	0.020	0.009
	LxD	0.216	0.007	0.013	0.022

Conclusions and Discussion

Supplementation of sows in late gestation with TSAA levels above NRC (3) recommendation improved colostrum nutrient content. The use of OH-Met in late gestation diet, led increasing Met, Cys and Lys better than DL-Met in colostrum. As Cys is related to redox control through glutathione synthesis it might be associated with the ability of piglets to better cope with immunological stress (2). Met supplementation reduced level of pro-inflammatory cytokines and also the hepatic injury produced by LPS challenge. In conclusion, supplementation of Met 25% above the NRC (2012) recommended level in sow and piglet diets, particularly as OH-Met, leads to significant improvement of milk quality and improved piglet ability to cope with LPS challenge.

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Copper and zinc association in the diet of growing and finishing pigs improves carcass quality

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Introduction

The prolonged use of Zn and Cu in pharmacological level such as ZnO and $CuSO_4$ may causes environment pollution and selective pressure on antimicrobial resistant bacteria (2). On the other hand, hydroxychloride minerals source (CHC and ZHC) are less reactive, increase feed stability, bioavailability and performance of pigs (1,6). The supplementation of these minerals in the diet of growing and finishing pigs can improve carcass and meat quality due to pro-oxidant actions (4). Thus, the present study aimed to investigate the effects of the association of Cu and Zn hydroxychloride minerals on performance, carcass and meat quality.

Materials and Methods

A total of 256 pigs were distributed in 4 treatments with 8 pen/treatment. The diets from 70 to 112 days of age (d) were T1: 100 mg/kg $CuSO_4$ + 80 mg/kg ZnO; T2: 150 mg/kg $CuSO_4$ + 80 mg/kg ZnO; T3: 100 mg/kg CHC + 80 mg/kg ZHC; T4: 150 mg/kg CHC + 80 mg/kg ZHC; and from 112 to 154 d T1: 90 mg/kg $CuSO_4$ + 70 mg/kg ZnO; T2: 150 mg/kg $CuSO_4$ + 70 mg/kg ZnO; T3: 90 mg/kg CHC + 70 mg/kg ZHC; T4: 150 mg/kg CHC + 70 mg/kg ZHC. Flavomycin was associated with treatments with low Cu levels. Performance was assessed by body weight (BW) at 70, 84, 91, 98, 112, 126, 140 and 154 d, as well as ADG, ADFI and G:F between these periods. At 154 d the animals were slaughtered and carcass was evaluated (hot and cold carcass weight, length, backfat thickness, loin eye area, subcutaneous fat thickness, loin depth, carcass and meat yield). Also, meat quality (pH and temperature, color, drip loss, cooking loss and shear force). The design was in randomized blocks. The treatment effect was analyzed by the PDIFF test and were considered significant when $p<0.05$.

Results and Discussion

The pigs supplemented with CHC and ZHC (T3 and T4) had higher BW at 154 d than T2 ($p<0.05$), and similar between them. This is probably due to the crystalline structure of hydroxychloride minerals that allows a slower release throughout the intestine, resulting in more efficient absorption (5). Most studies suggest that Cu enhances performance during early growing and finishing phases with little or no response during the late finishing phase (3). In the present study, it was observed that animals supplemented with hydroxychloride sources continued to grow, especially in the final stage of the finishing phase. The animals supplemented with hydroxychloride sources showed similar body weight to those receiving T1 throughout the experimental period, demonstrating that the use of antimicrobial as a performance growth promoter maybe could be removed from the diet. Also, CHC and ZHC increase final live weight, it also led to an increase in hot and cold carcass weight ($p<0.05$, Figure 1). Supplementation with hydroxychloride and flavomycin sources tended to have a larger loin eye area ($p=0.059$) compared to the other animals. In addition, supplementation with hydroxychloride sources without flavomycin resulted in higher carcass length ($p<0.05$) and tendency to lower cooking loss percentage ($p=0.08$), evidencing possible benefits of supplementation with hydroxyl sources in meat quality.

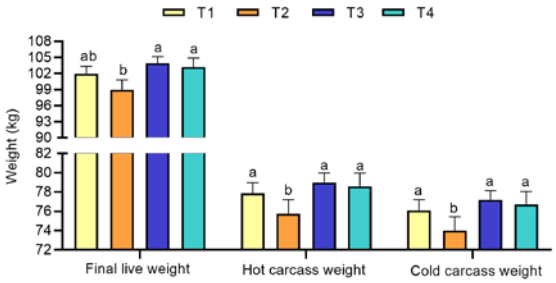

Figure 1. Final live weight, hot and cold carcass weight.
[ab]Bars without a common superscript letter differ ($P<0.05$)

Conclusions

The association of Copper and Zinc hydroxychloride improved final live weight, benefited some carcass characteristics and evidenced possible benefits in meat quality. More studies are necessary to evaluate the withdrawal of the flavomycin antimicrobial as an antibiotic growth promoter from the diet.

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To Trouw Nutrition and CAPES.

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Different periods of ractopamine supplementation for heavy gilts

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Introduction

Ractopamine (RAC) is a ß-adrenergic agonist and has been widely used in pig farming to improve the performance and carcass characteristics, regardless of slaughter weight. The main effects of this additive on metabolism are related to the inhibition of lipogenesis, increased lipolytic activity and muscle synthesis (1). According to the literature, the maximum effect of this additive has been obtained between 2 to 5 weeks of dietary supplementation (2, 3). However, the response progressively declines over the remaining days of the feeding period. The decrease in response to RAC has been attributed to down-regulation or desensitization of β-receptors when RAC is fed for longer periods (4). In addition, another important factor to be considered is the period of RAC supply, with supplementation in 4 weeks before slaughter being usual for animals weighing close to 100kg. However, the best period of RAC supply for pigs slaughtered at heavier weight is not yet established in the literature. Therefore, the objective of this study was to evaluate the effects of periods of RAC supply for heavy gilts on performance.

Materials and Methods

The study was performed in the pig breeding sector of the Department of Animal Science of Federal University of Ceará, Fortaleza, Brazil. A total of 32 gilts with an initial weight of 69.05 ± 6.10 kg were distributed in a randomized block design with 4 treatments, 8 replicates and 1 animal per experimental unit. The criteria adopted for the formation of the blocks were the initial weight and backfat thickness of the gilts. The treatments consisted of a diet without RAC supplementation (T1); diet with 10ppm of RAC from 142 to 170 days of age (T2); diet with 10ppm of RAC from 128 to 142 and 156 to 170 days of age (T3); diet with 10ppm of RAC from 100 to 128 and 142 to 170 days of age (T4). The diets were formulated considering the nutritional composition of feedstuffs and the nutritional requirements of gilts, according to Rostagno et al. (5). At the beginning and end of each period, gilts were weighed, as well as the amount of feed supplied and refusals daily collected. From the data obtained, performance parameters were analyzed regarding daily feed intake (DFI), daily weight gain (DWG) and feed conversion (FC). Data were submitted to analysis of variance by General Linear Models (GLM) procedure, and means were compared by Student–Newman–Keuls's test at 5% of probability by Statiscal Analysis System (SAS® – University Edition).

Results and Discussion

There was a significant difference ($P< 0.05$) among treatments on DWG and FC (Table 1). Dietary supplementation of ractopamine for heavy gilts provided a better DWG in relation to animals fed the control diet (T1). For FC, T4 had the best result, followed by T3 and T2 that did not differ from each other. In this sense, the data suggest that the interspersed supply of RAC for 28 days may have promoted a new sensitization in β receptors, which provided the best performance of heavy gilts.

Table 1. Effects of dietary supplementation of ractopamine for finishing gilts on performance

Treatments	Parameters		
	DFI, kg	DWG, kg	FC
T1	2.93	0.93[b]	3.13[a]
T2	2.93	1.02[a]	2.87[b]
T3	2.96	1.04[a]	2.88[b]
T4	2.90	1.06[a]	2.74[c]
CV (%)[1]	4.85	4.21	3.41
P-value	0.854	<.0001	<.0001

[1]Coefficient of variation. Means followed by different letters on the same column differ from each other by Student-Newman-Keuls's test ($P< 0.05$).

Conclusions

The dietary supply of 10 ppm of RAC for heavy gilts improves the DWG and FC of these animals. The best period of ractopamine supplementation for heavy gilts is from 100 to 128 days of age, followed by a period of 14 days without the use of this additive, plus a supply of ractopamine from 142 to 170 days of age.

Acknowledgments

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Effects of dietary supplementation of sodium butyrate for mixed-parity sows on litter performance

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Introduction

Butyric acid is a short chain fatty acid produced by microbial fermentation in the large intestine of the animals (1). In the gastrointestinal tract, it acts by inhibiting the colonization and proliferation of pathogenic microorganisms, improving the integrity of intestinal mucosa and, consequently, absorption of nutrients, which can significantly enhance animal performance (2). Because of its characteristics at room temperature, its use in pig diets often occurs as sodium butyrate (SB), because SB is more stable and less volatile. Several studies have demonstrated the effects of this additive for pigs, especially in piglet diets during the nursery phase (2, 3). However, studies evaluating the effects of SB supplementation for lactating sows on their litter are scarce. In addition, no information about the different forms of sodium butyrate supply (coated vs. uncoated) is provided. Therefore, the objective of this study was to evaluate the coated and uncoated sodium butyrate supplementation in diets for mixed-parity sows during lactation on performance of suckling piglets.

Materials and Methods

A total of 192 mixed-parity sows (Landrace × Large White) were distributed in a completely randomized design in a 4 × 4 factorial arrangement, considering 4 parity orders (1st, 2nd, 3rd and 4th, 5th to 7th) and 4 diets, totaling 16 treatments with 12 replicates each, considering each sow and their litter as experimental unit. The following experimental diets were applied: control diet without sodium butyrate; diet with 0.1% and 0.2% coated sodium butyrate; and diet with 0.066% uncoated sodium butyrate. The piglets were weighed 48h after farrowing (LW48) and at weaning (LWW). From this data, litter daily weight gain (DWG) was analyzed. Data were submitted to analysis of variance by General Linear Models (GLM) procedure, and means were compared by Tukey test at 5% of probability by Statiscal Analysis System (SAS®).

Results and Discussion

No interaction was observed ($P > 0.05$) between the supplementation of SB and the different parity orders on the performance of litters (Table 1). The supplementation of SB did not influence the performance of litters ($P > 0.05$), corroborating with Jang et al. (4), which did not observed difference in piglet development from sows supplemented with SB during lactation. However, for LW48 and LWW and, consequently, for the DWG elicited a response among the parity orders ($P < 0.05$). The effects of parity orders on litter performance are in accordance with Martins et al. (5), whose affirmed that the lower DWG of piglets from primiparous sows is due to the lower intake of the main nutrients present in the milk of these sows.

Table 1. Litter performance from mixed-parity sows supplemented with sodium butyrate during lactation

	LW48	LWW	DWG
Supplementation			
Control	19.09	73.70	2.59
0.1% Coated SB	19.24	74.67	2.55
0.2% Coated SB	18.72	74.75	2.59
0.066% Uncoated SB	18.96	74.48	2.65
Parity order (PO)			
1	17.21[b]	69.54[b]	2.36[b]
2	19.27[ab]	75.44[ab]	2.65[a]
3 and 4	19.00[ab]	75.07[ab]	2.64[a]
5 to 7	20.51[a]	77.55[a]	2.71[a]
CV[1]	17.39	13.97	15.64
P-value			
SB	0.914	0.969	0.730
PO	<.0001	0.001	0.004
SB*PO	0.063	0.153	0.087

[1]Coefficient of variation. Means followed by different letters on the same column differ from each other by Tukey test ($P < 0.05$).

Conclusions

Dietary supplementation of coated and uncoated sodium butyrate for lactating sows does not influence their litter performance. Primiparous sows have lighter litter when compared to sows of 5th to 7th parity order.

Acknowledgments

CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico).

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Profitability in piglet production with immunity and animal health

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Introduction

The weaning happens when the piglets still haven´t mature immunology. The immune system is been stimulated and the high activation costs energy to the animal. The resulting effects are reduction of appetite and low development. Growth promoters are used to minimize the damage and improve piglet´s performance, but their use is decreasing and the demand to alternatives is increasing. A linear 1,3 β-glucan naturally derived from algae *Euglena gracilis* (ALE) shows to be a solution during the immunity challenge period and an alternative to growth promoter program. The objective of this trial was to evaluate the use of a 1,3 β-glucan as a replacement for growth promoter programs post-weaning.

Materials and Methods

Weaning piglets with 23 days of age and an average weight of 6.7 kg were assigned to two treatments: Control and ALE. Each treatment was composed by 120 piglets (10 replicates with 12 pigs/pen, divided in males and females and blocked by initial body weight). The trial period was 2 weeks post-weaning, and it was divided in two phases: Pre-Starter I (7 days) and Pre-Starter II (7 days). The control group used Flavomycin as a growth promoter program and the ALE group used the ALE product in replacement of growth promoter. The diets were formulated according to piglets´ requirements during the respective phases, with same nutritional levels and ingredients, except for growth promoters and ALE product. The animals were weighted after every phase and the feed consumption computed. The parameters evaluated were body weight (BW), average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR). Diet costs were evaluated to calculate the return on investment (ROI) to compare growth promoter *vs* ALE use. The data was compared by Tukey test and analyzed using SAS program (9.4).

Results

The performance data is shown in Table 1. In the first week after weaning (7 days), the ALE treatment had the best performance. The piglets gained more than 27% weight per day ($p<0.01$) in comparison to control group and had better consumption ($p<0.01$) with the less FC ($p<0.01$) in the Pre-starter I phase. During the second phase, ALE continues improving GPD ($p<0.01$) and ADFI ($p< 0.01$).

Conclusions and Discussion

In the first week after weaning, when the piglets feel the weaning stress and immunity is low, treatment with ALE had the best performance. The piglets gained more than 27% weight per day in comparison to control group, indicating the action of ALE in swine immunity, improving health and performance. Normally, about 10 days are expected for animals to recover intestinal integrity and microbiota balance, normalizing feed consumption. ALE group also promoted better consumption with less FC.

During the second phase (14 days), when the piglets continue to be susceptible to diseases, ALE continues to improve GPD and ADFI. The use of ALE promove more 250 g/piglet (+23% increase in weigh gain) in post-weaning.

Table 1. The effect of ALE in replacement of growth promoter program on body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), feed conversion rate (FCR) and weight gain

	Control	ALE	*p-value*
Initial BW (kg)	6.77	6.77	
7 days BW (kg)	8.12[b]	8.48[a]	*0.0003*
ADG 7 days (g)	192[b]	244[a]	*<0.0001*
ADFI 7 days (g)	278[b]	302[a]	*0.0072*
FCR 7 days	1.45[a]	1.24[a]	*<0.0001*
14 days BW (kg)	10.64	10.89	*0.1143*
ADG 14 days (g)	276[b]	294[a]	*0.0032*
ADFI 14 days (g)	383[b]	414[a]	*0.0014*
FCR 14 days	1.39	1.43	*0.8601*
Weight gain (kg)	3.87	4.12	

[a,b] Different letters in the same line indicate a significant difference between treatments ($p<0.01$)

Related to economic analysis of ALE as a replacement for the growth promoter program, the parameters feed consumption per animal per phase and feed cost per phase were evaluated to calculate the return on investment (ROI). The use of ALE had better benefit and resulted in a ROI of 2:1.

These results justify the investment in additive that show better immunity and animal health status in post-weaning, with profitability to the production of piglets.

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Calcium butyrate slow release, intestinal energy source, improves piglet´s performance in post weaning and profitability to nursery phase

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Introduction

Weaning is a critical period to piglets, the immediate effect is a dramatic reduction in feed intake with reduced growth performance. The low feed intake may contribute to intestinal inflammation and adversely affect villous height and crypt depth. The small intestine and its mucosa lose 20–30% of their relative weight during the first two days post weaning, while regeneration will need 5 to 10 days for full recovery. Intestinal crypt cell proliferation and rates of cell migration appear to depend strongly on energy availability during the post weaning period. Butyric acid is a short chain fatty acid known to serve as energy source for all cells, but also supports cell proliferation and improves the development, maturation and growth of the intestinal epithelium. The objective of this study was to evaluate the effect of an encapsulated source of calcium butyrate slow release (BUTIP), which allows its release throughout the intestinal tract, supporting the development and growth of the animal during post-weaning stress when piglet's gastrointestinal tract needs to be recovered.

Materials and Methods

Weaning piglets with 23 days of age and an average weight of 6.7 kg were assigned to two treatments: Control and BUTIP. Each treatment was composed by 120 piglets (10 replicates with 12 pigs/pen, divided in males and females and blocked by initial body weight). The trial period was 49 days and it was divided in four phases: Pre-Starter I (7 days), Pre-Starter II (7 days), Starter I (7 days) and Starter II (28 days). The diets were formulated according to piglet´s requirements during the respective phases, with same nutritional levels and ingredients. In the BUTIP treatment, the inclusion of the product was 1.0 kg/ton in Pre-Starter I feed, 750 g/ton in Pre-Starter II feed, 500 g/ton in the Starter I feed and no inclusion in the Starter II feed. The animals were weighted after every phase and the feed consumption computed. The parameters evaluated were body weight (BW), average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR). Diet costs were evaluated to calculate the return on investment (ROI) resulting from the use of BUTIP. The data was compared by Tukey test and analyzed using SAS program (9.4).

Results

The performance data is shown in Table 1. The BUTIP group had the best performance in the post weaning week, since piglets gained more 22% ADG ($p<0.01$), 7% ADFI ($p<0.01$) and consequently better FCR ($p<0.01$). The positive effect in ADG and ADFI continued during the Pre-Starter II phase ($p<0.05$). This effect in initial phases had a positive impact the total growth period, being the total ADG better (p 0.07) and best final body weight, 30.21 vs 31.00 kg (p 0.07). Considering economic analysis, the parameters feed consumption per animal per phase and feed cost per phase were evaluated to calculate the ROI. The use of BUTIP in piglet's diet in nursery phase had better benefit and resulted in a ROI of 19:1.

Table 1. The effect of BUTIP on initial body weight (IBW), average daily gain (ADG), average daily feed intake (ADFI), feed conversion rate (FCR) and average final body weight (FBW)

	Control	**BUTIP**	***p-value***
IBW (kg)	6.77	6.77	
Pre-starter I phase			
ADG (g)	192[b]	235[a]	*0.0001*
ADFI (g)	278[b]	297[a]	*0.0198*
FCR	1.47[b]	1.28[a]	*0.0040*
Pre-starter II phase			
ADG (g)	276[b]	301[a]	*0.0054*
ADFI (g)	383[b]	400[a]	*0.0312*
FCR	1.39	1.34	*0.6629*
Starter I phase			
ADG (g)	344	352	*0.3223*
ADFI (g)	468	471	*0.7702*
FCR	1.36	1.34	*0.3966*
Starter II phase			
ADG (g)	478	494	*0.0737*
ADFI (g)	755	764	*0.5391*
FCR	1.58	1.55	*0.0761*
FBW (kg)	30.21	31.00	*0.0739*

[a,b] Different letters in the same line indicate a significant difference between treatments ($p<0.05$)

Conclusions and Discussion

The immediate effect of weaning is reduction in feed intake and growth performance. However, the BUTIP group showed the best performance in post-weaning. The BUTIP action in the initial phase had positive effect in the total period, since piglets of BUTIP group had the best final weight, with almost 800 g gain in the final weight in comparison to control group. BUTIP in piglet´s diet in post-weaning period promotes gastrointestinal tract recover with positive consequence in performance data. This effect promotes best weight gain in final nursery phase with good profitability.

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Effects of dietary supplementation of guanidinoacetic acid for sows and their progenies on piglet performance at nursery phase

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Introduction

The current genetic selection programs used in pig farming seek prolific sows, with potential to produce numerous litters. However, this advance has generated some negative aspects, such as piglets with low birth weight. In the subsequent phases, these animals generally present worse performance, with consequent increase in slaughter age (1). Aiming to solve these inherent failures in pig farming advances, many researches involving the nutrition of sows and their litter have been performed, with focus on nutritional additives, such as the guanidinoacetic acid (GAA). GAA acts as an arginine-sparing compound, which can be used in others body functions. The increased availability of arginine in the body can promote a greater vascularization in the placenta and mammary gland of pregnant and lactating sows, through the synthesis of nitric oxide, providing greater transfer of nutrients to fetuses (2, 3). Furthermore, arginine acts directly on protein turnover, promoting a greater anabolism and attenuating catabolism (4). Therefore, the objective of this study was to evaluate the effects of dietary supplementation of 0.1% GAA for sows and their progenies on piglets' performance at nursery phase.

Materials and Methods

A total of 80 multiparous sows (Landrace×Large White), were selected considering body weight and backfat thickness. After 24 h of artificial insemination, sows were randomly distributed between 2 dietary treatments: control diet and diet supplemented with 0.1% GAA. Sows were fed with the dietary treatments during the gestation and lactation. At 23 days of age, the litters were weaned, weighed and from the initial dietary treatments were distributed in a randomized block design in a 2×2 factorial arrangement, considering 2 dietary treatments for sows during gestation and lactation (control diet with 0 or 0.1% GAA) and 2 dietary treatments for piglets at nursery phase (control diet with 0 or 0.1% GAA), totaling 4 treatments with 6 replicates each, considering the pen with 40 animals as experimental unit. The criterion adopted for the formation of the blocks was the initial weight of the piglets. During the experimental period, piglets were weighed, as well as the amount of feed supplied and refusals daily collected. From the data obtained, performance parameters were analyzed regarding average daily feed intake (ADFI), average daily gain (ADG) and feed conversion (FC). Data were submitted to analysis of variance by General Linear Models (GLM) procedure, and means were compared by Tukey test at 5% of probability by Statiscal Analysis System (SAS®).

Results and Discussion

There were no interaction (P>0.05) between GAA supplementation for sows and for their progenies on ADFI, ADG and FC of piglets at nursery phase (Table 1). In addition, no significant differences were observed (P>0.05) on piglets performance considering GAA dietary supplementation for sows or piglets. The lack of more evident results of GAA dietary supplementation on ADG and FC in the present study may have occurred because the diets provided to the piglets at nursery phase meet or exceed their arginine requirements, since contained feedstuffs with high levels of these amino acids, such as meat meal and dairy products. In addition, no differences were observed in ADFI of piglets, because GAA does not have flavoring properties capable of stimulating a greater intake by the animals.

Table 1. Effects of dietary supplementation of GAA for sows and their progeny on piglet performance

GAA supply	ADFI, kg	ADG, kg	FC
Sows, 0%	0.547	0.410	1.34
Sows, 0.1%	0.569	0.425	1.36
Piglets, 0%	0.558	0.425	1.33
Piglets, 0.1%	0.559	0.410	1.37
CV[1]	4.99	4.33	3.72
P-value			
Sows	0.501	0.442	0.173
Piglets	0.709	0.899	0.679
Sows*Piglets	0.178	0.364	0.601

[1]Coefficient of variation. Means followed by different letters on the same column differ from each other by Tukey test (P<0.05).

Conclusion

Dietary supplementation of 0.1% GAA for sows and their progenies does not influence the performance of piglets at nursery phase.

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Effects of dietary supplementation of guanidinoacetic acid on creatinine and creatine kinase levels of piglets at nursery phase

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Introduction

Guanidinoacetic acid (GAA) is a metabolic intermediate synthesized from the amino acids Gly and Arg. When supplied in the diet, GAA is rapidly absorbed in the gastrointestinal tract, being transported to the liver, where it receives a methyl group of S-adenosyl-L-methionine, producing creatine (1). A higher content of this protein in the body can indirectly promote an increase in muscle mass, because it stimulates an influx of water into the muscle cells, inducing protein synthesis and reducing proteolysis (1, 2, 3). Compared with creatine, GAA is more stable and less expensive, which has led to the idea that perhaps GAA could be a substitute for dietary creatine in swine nutrition (4). Diets with substances that increase protein deposition, such as creatine, lead to increased blood levels of creatinine (CRE) and creatine kinase (CK). These molecules are often described as important indirect muscle markers and also as indicative of pig performance (5, 6). Therefore, the objective of this study was to evaluate the effects of dietary supplementation of 0.1% GAA on blood levels of CRE and CK of piglets at nursery phase.

Materials and Methods

The study was performed in the facilities of a commercial farm, located in the Southern region of Brazil, in Santa Catarina state. A total of 960 piglets weaned at 23 days of age were distributed in a randomized block design with 2 treatments and 12 replicates, considering the pen with 40 animals as experimental unit. The criterion adopted for the formation of the blocks was the initial weight of the piglets, with average weight of the light and heavy piglets of 4.9 ± 0.2 kg and 6.8 ± 0.3 kg, respectively. The treatments consisted of control diet and diet supplemented with 0.1% GAA. During the nursery phase, water and feed were available *ad libitum* to piglets. Feed were formulated considering the nutritional composition of feedstuffs and the nutritional requirements of piglets, according to Rostagno et al. (7). Blood samples were collected in three animals per experimental unit, based on the piglets mean weight of each replicate at 23 and 63 days of age. A total of 5 ml of blood were collected in each animal by puncturing the jugular vein, using sterilized needles and plain tubes. After collection, samples were centrifuged and the serum obtained was stored in Eppendorf tubes at -20 °C. The methodology employed for the determination of CRE and CK was enzymatic colorimetric, using commercial kits (Labtest Diagnóstica S.A.). Data were submitted to analysis of variance by General Linear Models (GLM) procedure, and means were compared by Tukey test at 5% of probability by Statiscal Analysis System (SAS®).

Results and Discussion

There were no effects (P>0.05) of dietary supplementation of GAA on serum concentrations of CRE and CK of the piglets at nursery phase (Table 1). The absence of significant differences in GAA supplementation on creatinine and creatine kinase levels may have occurred because the requirement in amino acids was being met for maximum muscle deposition. However, it was observed that all values are within the range expected for pigs, which are from 1.0 to 2.7 mg/dL and 2.4 to 22.5 IU/L for creatinine and creatine kinase, respectively (8).

Table 1. Effects of dietary supplementation of GAA on blood parameters of piglets at nursery phase.

Parameters	0% GAA	0.1% GAA	CV (%)	*P-value*
23 days of age				
CRE, mg/dL	1.12	1.16	3.87	0.274
CK, UI/L	3.61	3.66	2.21	0.136
63 days of age				
CRE, mg/dL	1.46	1.50	3.90	0.362
CK, UI/L	6.80	6.88	2.64	0.212

[1]Coefficient of variation. Means followed by different letters on the same line differ from each other by Tukey test (P<0.05).

Conclusion

Dietary supplementation of 0.1% GAA does not influence the blood levels of creatinine and creatine kinase of piglets at nursery phase.

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Organic acids in the drinking water for weaned piglets improves the water intake and antioxidant capacity in jejunum

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Introduction

Weaning is considered the most challenging period of pig's life. The combination of multiple stressors at weaning lead to lower feed intake and diarrhea resulting in poor growth and increased susceptibility to infectious diseases (1). Therefore, the objective of this research was to investigate the effect of using organic acids in the drinking water on performance and general health of weaned piglets.

Materials and Methods

One hundred twenty-two weaned piglets (5.17 kg, ±20 d of age) were assigned in a completely randomized block design to one of four treatments with seven replicates: negative control (NC, basal diet), positive control (PC, NC+ 150/120/80/80ppm of colistin), and a synergistic blend of free and buffered organic acids (OA) (OA1, 1.0 L OA/1000 L water, or OA2, 2.0 L OA/1000 L water). Twice a day, the water usage (WU)/pen was recorded, and the fecal score analyzed once a day to calculate the diarrhea incidence. On days 3 and 4 all piglets were oral inoculated with *Escherichia coli* K88$^+$. On day 9, 28 piglets (one per pen closest to the pen mean BW) were euthanized for collection of jejunum (evaluation of mucosal integrity, quantification of antioxidant enzymes) and cecal content volatile fatty acids (VFA) analysis and *Bifidobacteria* and *Lactobacillus* CFU counting).

All variables measured were tested for normality. The data was analyzed using the MIXED procedure of SAS (SAS Institute Inc.) as a randomized complete block design (initial BW). Pen was considered as the experimental unit for the growth performance and water usage analysis. For the intestinal health variables, each animal sampled was considered one experimental unit. All data were reported as least squares means and compared by Tukey test. Results were considered significantly different if $P < 0.05$ and trends were discussed if $P>0.05<0.10$.

Results

No differences among treatments were found for ADG and ADFI, resulting in no differences in feed efficiency as well ($P>0.05$, Table 1). The inclusion of OA in the water (OA2) increased water usage when compared to NC and PC for all weeks of evaluation and for the overall period, respectively ($P<0.05$). No statistical difference was detected for diarrhea incidence, however, OA1 had a numerical reduction of 27% when compared to NC on week 2 (30.6% vs 42.9%, respectively). No differences were found for mucosal integrity and CFU counting ($P>0.05$). OA1 tended to have greater concentration of acetic, propionic acid and total VFA concentration when compared to OA2 and did not differ from NC and PC ($P<0.10$). OA1 had greatest concentration of superoxide dismutase ($P<0.0001$). NC had the smaller concentration of catalase with OA1 and OA2 being intermediates ($P=0.037$). OA1 tended to have greater concentration of glutathione transferases when compared to PC ($P=0.094$). No differences were found for glutathione and lipid peroxidation in jejunum ($P>0.05$).

Conclusions and Discussion

The positive effects of OA in post-weaning diets has been attributed to its antimicrobial activity and the reduction in luminal pH (2).

Table 1. Effect of organic acids (OA) in the drinking water on nursery piglets water usage (WU) and feed efficiency (Gain:Feed, G:F).

Item	Treatments[1]				P-Value
	NC	PC	OA1	OA2	
0 to 7 d					
BW d 7, kg	5.31	5.38	5.31	5.29	0.992
G:F	0.28	0.39	0.21	0.33	0.771
WU, L/day	1.03^b	1.28^{ab}	1.43^{ab}	1.80^a	0.045
8 to 14 d					
BW d 14, kg	6.33	6.30	6.27	6.16	0.970
G:F	0.47	0.49	0.49	0.44	0.838
WU, L/day	1.24^b	1.44^b	1.82^b	3.00^a	<0.01
0 to 41 d					
BW d 41, kg	16.81	17.96	17.48	18.20	0.484
G:F	0.59	0.59	0.58	0.63	0.131
WU, L/day	1.78^b	2.19^b	2.26^{ab}	2.79^a	0.001

^{a,b,c}Within a row, means with different letters differ by Tukey test ($P < 0.05$). [1]NC: Negative Control; PC: Positive Control (NC + 150; 120; 80; and 80 mg/kg of colistin sulphate in each phase step-down); OA1: (NC + 1.0 L OA/1000 L water); OA2: (NC + 2.0 L OA//1000 L water).

Results from the present study suggest that OA in the drink water enhance the water usage and provides equally growth performance when compared to antibiotic utilization. Improving water intake is important for hydration status as it is related to improvement in feed intake. Organic acids in the drinking water for weaned piglets, as an alternative to antibiotics, may reduce the diarrhea incidence and improve the antioxidant capacity in the jejunum.

Acknowledgments

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Bacillus strains reduce viremia and pathogenicity in a porcine reproductive and respiratory syndrome virus and *Salmonella choleraesuis* coinfection model

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Introduction

Weaning imposes a major stress, resulting in reduced feed intake and growth, that combined with the presence of viral and bacterial pathogens contribute to increased morbidity and mortality. Coinfection of porcine reproductive and respiratory syndrome virus (PRRSv) and *Salmonella choleraesuis* in swine has been associated with increased clinical effects of salmonellosis in the field, including septicemic salmonellosis and severe pneumonia (1). Indeed, PRRSv has been shown to render pigs more susceptible to S. *choleraesuis* septicemia and these two pathogens act synergistically to produce a more severe systemic and respiratory disease (2). Experimental coinfection of weanling pigs with PRRSv and S. *choleraesuis* was selected as a model to examine the potential mitigating effect of a direct-fed microbial (DFM) on their combined pathogenicity.

Materials and Methods

Twenty-one-day-old pigs (7.49 ± 1.27 kg BW) free from PRRSv and *Salmonella* were randomly allotted into two groups (n=11 per group) and fed a common nursery diet without or with supplementation of DFM (ProVent® ECL, United Animal Health, Inc.). On d 14, both groups were orally challenged with 10^9 cfu of *S. choleraesuis* followed by intranasal challenge with 2×10^4 $TCID_{50}$ of PRRSv on d 17. A third group (n=4) served as a non-challenged and non-supplemented control. The animals were monitored daily for the presence of clinical signs. Serum samples were collected at the indicated time points, and on d 23-24, a full necropsy was performed to examine respiratory and intestinal tissues for the presence of pathological lesions. Lung gross pathology was scored based on a standard scoring system. Samples from the lung, bronchial lymph node, and ileocolic lymph node (ICLN) were examined for the presence of *Salmonella* using quantitative bacteriology. The extent of viremia was determined by $TCID_{50}$ assay using pig alveolar macrophages. The extent of immune gene expression in blood cells was determined by real-time reverse transcription PCR, using the comparative threshold cycle (Ct) method and the formula $2^{-\Delta\Delta Ct}$, with the GAPDH gene as the reference housekeeping gene. Differences on the extent of gross pathology and fold change gene expression relative to control was analyzed by ANOVA (Minitab 17.1.0) and level of cytokines by either one-tailed unpaired t-test or Mann-Whitney test (Prism 8.0.2).

Results and Discussion

Coinfection elicited only minor clinical signs of disease but reduced ($P < 0.01$) ADG regardless of DFM supplementation. *Salmonella* was isolated from the ICLN of all challenged pigs with no difference in the amount of *Salmonella* recovered (cfu/gm) regardless of DFM supplementation. Provision of DFM reduced (60 vs 91%; $P < 0.05$) *Salmonella* colonization of the lung and associated lymphoid tissue, and reduced (55 vs 84%; $P < 0.05$) the extent and severity of gross lung pathology. Supplementation with DFM reduced viremia (3.08 vs 4.09 $TCID_{50}$/ml (log10); $P < 0.05$) and the severity of the *Salmonella*-induced exudative inflammation, as indicated by a lower frequency (50 vs 82%; $P < 0.05$) of pigs exhibiting fibrinous ascites production. At necropsy, concentrations of IL-1 (287 vs 140 pg/ml) and IL-8 (1,700 vs 600 pg/ml) were elevated ($P < 0.05$) in lung lavage fluids collected from DFM-supplemented pigs, which was accompanied by increased expression (25- vs 18-fold-change; $P < 0.05$) in white blood cells of the immunoreceptor TREM-1. Similar results were observed in DFM-treated pigs only challenged with *Salmonella*.

Conclusions

Diet supplementation with DFM reduced the rate of spread of *Salmonella* from the intestinal tract to the lung, reduced the inflammatory response to PRRSv-*Salmonella* co-infection, and reduced the level of viremia. Changes in inflammatory cytokine production and increased expression of TREM-1 suggest that feeding of *Bacillus* strains promotes a modulated pro-inflammatory innate immune response. These results indicate that DFM can exert a mitigating effect on the systemic spread of *Salmonella*, as well as the pathogenic synergy resulting from a PRRSv and *Salmonella* coinfection by reducing viremia and modulating the inflammatory response in the lung.

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The impact of an agglomerate of dietary sodium diformate and monolaurate on the faecal microbiome of lactating sows

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Introduction

Gut health is increasingly being shown to be effective against intestinal pathogens, a strategy that has only really been made possible through the removal of antibiotic growth promoters in feed. Creating and maintaining a healthy intestinal environment has become essential to productivity and food safety programmes alike (4). The application of organic acids and their salts to diets for pigs has been studied extensively over decades. They are especially effective in maintaining growth performance, since the ban on antibiotic growth promoters came into effect in Europe and this strategy is now applied in many countries worldwide. Numerous trials have demonstrated the mode and magnitude of action of organic acids in feed for pigs and has established effective doses for piglets, fattening pigs and sows, among them the use of diformates (6). In addition it is known that monolaurate has a strong antibacterial impact against various Gram-positive bacteria (1), making it a promising candidate as an additive or as an alternative to antibiotics for treatment of different diseases (7). The gut microbiome, which is also represented to a great extent in the faecal microbiome, is a community of host-associated symbiotic microbes that fulfils several important roles in host metabolism and immune function (3). Therefore, an increased understanding of the gut microbiota has the potential to have an impact on nutrition, feed efficiency and general health in pig farming (8). The current study therefore investigates the impact of an agglomerate of sodium diformate and monolaurate on its effect on the faecal microbiome of sows during lactation.

Materials and Methods

Multiparous sows on a commercial farm in Australia were fed either a lactation diet, which contained a regular acidifier at 0.8% as control – or a test diet, which contained 1% of a sodium diformate-monolaurate agglomerate (traded as Formi 3G, ADDCON) instead. The diets were fed from the 90th day of gestation until weaning. Faecal samples (n=24) were collected on the 5th day of lactation. The reduction rates (%) of streptococci and clostridia on their relative abundance of the overall faecal microbiome were measured via the application of New Generation Sequencing (NGS) using Microgenetix. Data were analysed using the t-test and the Wilcoxon-Mann-Whitney test. A significance level of 0.05 was used in all tests.

Results

Mean abundance rates of specific pathogenic bacteria are shown in Table 1. The three main effects of the test diet were a significant (p<0.018) reduction of the relative abundance of clostridia spp. by 42.2% and of streptococci spp. by 42.5% (p<0.013), while the number of streptococci positive faecal samples dropped significantly (p<0.002) by 79.6%.

Table 1. Relative abundance (%) of clostridia spp. and streptococci spp. in the sow faecal microbiome

Diet	Control	Formi3G	Diff.	*p*
Clostridia	19.2^{a}	11.2^{b}	-42.2	0.018
Streptoc.	3.2^{a}	1.8^{b}	-42.5	0.013

(a, b) Superscripts indicate statistically significant differences ($p \leq 0.05$).

Conclusions and Discussion

The addition of the agglomerate of sodium diformate and monolaurate caused a significant improvement of the health status of sows, based on their faecal microbiome. The impact against the Gram-positive streptococci is especially noteworthy. This is in full agreement with earlier trials in Europe (2, 5). The combined inclusion of diformate and monolaurate may therefore not only provide a healthy gut in sows, but might furthermore support a pork production chain with reduced zoonotic pathogen pressure. This will additionally help antibiotic reduction initiatives.

Acknowledgments

Microgenetix, Charles River Company, Melbourne, VIC, Australia

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Effect of dietary 25-hydroxycholecalciferol on growth performance and leg soundness in developing gilts

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Introduction

Vitamin D_3 (D_3) is a water-soluble vitamin and plays an important role for growth performance, productivity and bone strength in swine. Growth rate and bone strength in gilts are related to longevity, therefore increasing productivity of sows in a breeding herd. D3 converts into an active metabolite, 25-hydroxycholecalciferol (25-OHD_3), by liver. Normally, the 25-OHD_3 is added to feed for developing gilts only for short period of time, less than 50 days before breeding (1). However, little is known regarding long-term 25-OHD_3 supplementation. The aims of the present study were to compare the effect of long term 25-OHD_3 supplemented on growth performance and leg conformation soundness in developing gilts.

Materials and Methods

One-hundred-eighty gilts with from 10 weeks old were included. Gilts were randomly allotted in to three treatments (T1, T2, T3) and blocked by batches of age, 60 gilts each. Feed and water were provided ad libitum consumption until gilts were selected at 34 weeks of age. T1 feed was formulated to as basal diet with adding 2,000 IU D_3/kg o diet. T2 and T3 basal diets were fully replaced by 25 µg 25-OHD_3 of Bio-D and 50 µg ® 25-OHD_3 Hy-D® per kg of diet, respectively. Treatments were equivalent to 2,000 IU D_3/kg of diet. Body weight (BW), back fat (BF) thickness at 34 weeks old and average dairy feed intake (ADFI) were measured recorded and average daily weight gain (ADG) was calculated. Leg conformation soundness including forelimb and hindlimb (0-5) characteristics and locomotion (0-3) were scored by experienced researchers at 22 and 34 weeks old of gilts. The statistical analysis was conducted using *R* (v3.6.2).

Results

Table 1 is shown the effect of dietary 25-OHD_3 supplementation on growth performance, leg soundness and percentage of gilts selected as replacement gilts. Body weights at entry and at final were not statistically significant among. BF thickness and ADG in T2 were statistically higher (P=0.016 and P=0.020) than T1 and T3 but not statistically different in ADFI. Leg soundness locomotion score and percentage of gilts selected were not statistically significant were not found statistically significant.

Table 1. The effect of dietary 25-hydroxycholecalciferol supplementation on growth performance and leg soundness in replacement gilts

Parameter	Mean ± SEM[1]			P-value
	T1	T2	T3	
Age at entry, week	9.72 ± 0.10	9.72 ± 0.10	9.73 ± 0.10	0.997
BW at entry, kg	29.65 ± 0.70	29.75 ± 0.69	29.14 ± 0.71	0.808[1/]
BW at 22 weeks old, kg	84.67 ± 1.55	85.01 ± 1.45	83.33 ± 1.50	0.506[1/]
Age at final, week	33.88 ± 0.08	33.94 ± 0.08	33.99 ± 0.08	0.761
BW at final, kg,	140.92 ± 2.27	143.40 ± 2.19	140.60 ± 2.15	0.615[1/]
BF thickness at final, mm	13.89 ± 0.35[a]	14.78 ± 0.31[b]	13.78 ± 0.29[a]	0.016
ADFI, kg/pig	2.03 ± 0.02	2.04 ± 0.02	2.04 ± 0.01	0.617[2/]
ADG, g/day	720.56 ± 11.31[ab]	732.12 ± 11.46[a]	709.29 ± 11.20[b]	0.020
Locomotion score	1.05 ± 0.16	1.03 ± 0.15	1.29 ± 0.15	0.390[3/]
Percentage of gilt selected, %	65.00	72.13	72.88	0.587[4/]

[a, b] Means within same row with different superscripts differ (P< 0.05).
[1] SEM denoted standard error of mean;
[1/]Covariates appearing in the model were evaluated. Models were analyzed using: [2/]generalized estimating equations with repeated measurement; [3/]ordinal logistics regression; [4/]log-binomial regression.

Conclusions and Discussion

Dietary 25-OHD_3 supplementation reported with no negative effect to performance of preweaning piglets, nursery, growing pigs and sows (2, 3). In gilts, dietary 25-OHD_3 supplementation did not found different in performance, reproduction and bone status markers (4). In this study, long-term supplementation of 25-OHD_3 had no negative effects on leg soundness scores and percentage of gilts selected but had positive impact on increased ADG and BF thickness. In summary, 25-OHD_3 supplemented as long-term increased growth performance (ADG and BF thickness) in developing gilts. Hence, this may lead to increasing productivity of sows in subsequent parities.

Acknowledgments

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Isoquinoline Alkaloids supplementation reduces body weight loss of sows during lactation and increases IgG in colostrum

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Introduction

Plant extracts containing isoquinoline alkaloids (IQ) have demonstrated to have anti-inflammatory properties[1]. In pigs, IQ supplementation have shown to down regulate stress response and improve digestibility of nutrients[2,3]. The present experiment was conducted to test the hypothesis that supplementing sows with IQ during gestation would decrease stress at farrowing and improve colostrum quality, positively affecting piglets' health and performance.

Material & Methods

Twenty-four sows were blocked by parity and randomly allocated into three dietary groups: NC - basal diet without supplementation, IQ1 - 90 g/t IQ in the diet from day 80 of gestation (G) until day G110 and 150 g/t IQ from G110 (when entering maternity) until weaning (28 days), IQ2 - 150 g/t IQ from G110 until weaning. Blood was taken from sows five days before, during and one week after farrowing to measure cortisol, glucose and insulin. Colostrum was analyzed for protein, fat, IgA and IgG. Sow body weight, feed intake, backfat thickness and back-muscle thickness were monitored during the lactation period. In piglets, body weight gain was assessed weekly during the lactation period, as well as diarrhea score was monitored daily. Moreover, intestinal histomorphometry and gene expression of pro-inflammatory cytokines (IL6, IL10 and TNF-a) were assessed on day 5 post-weaning.

Results

IQ supplementation tended to reduce weight loss and backfat thickness loss during lactation ($p<0.10$). Five-days before farrowing, sows' blood glucose and insulin levels were lower in the IQ2 group ($p<0.05$) compared to the NC group. There were no differences in cortisol between treatments. Colostrum of IQ groups had a higher concentration of protein and IgG ($p<0.05$). Piglets' weigh at weaning was numerically higher in IQ1 and IQ2, and the physiological measurements were not different between groups.

	CON	IQ1	IQ2
Sows feed intake, second week of lactation (kg)	5.7	6.3	6.2
Body weight loss during lactation (kg)	43.2	33.6	28.8*
Backfat thickness loss during lactation (mm)	4.4	2.6*	3.4
Piglets weight at weaning (kg)	6.8	7.3	7.6
Piglets average daily gain during lactation (g/day)	191.9	211.4	225.9

*$p<0.10$.

Discussion & Conclusion

IQ seem to have their main effect on sow's metabolism, reducing body weight loss during lactation. Providing IQ to sows from entrance to the maternity barn might be enough to induce those effects. IQ improved colostrum quality increasing protein and IgG content, providing piglets with a better passive immunity.

Acknowledgements

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Use of Phosphatidylcholine to reduce fat deposition in fattening pigs

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Introduction

The prohibition of the ractopamine usage for pigs related to the exportation for other countries has a direct impact over the carcass performance of these animals (1). A natural source of phosphatidylcholine from herbs can stimulates genes associated with lipid deposition and glucose metabolism and it can interfere over the metabolism of fat deposition visceral and maybe also subcutaneous (2). The aim of this study is to relate the use of different dosages of an herb blend with 1,6 % of phosphatidylcholine with the carcass characteristics of late finishing animals.

Material and Methods

The experiment was performed at the experimental facilities of AKEI, a Research Centre in Fartura, SP-BR. A total of 280 pigs, PIC genetic (Matrix X Boar) were used in the trial. The animals were fed during 28 days before slaughter. The treatments were different dosages of phosphatidylcholine at 0, 250, 500 and 750 mg / Kg of a product (Biocholine FC) contending 1,6 % of phosphatidylcholine provided by Nutriquest Technofeed. It was evaluated performance and carcass parameters (each carcass was submitted to an electronic evaluation by Hennessy Grade Probe, Hennessy Grading Systems, Auckland, NZ, performed by trained personnel).

Results

There was no difference between the use of increasing levels of phosphatidylcholine and the final performance of the animals (Table 1).

Table 1. Performance of finishing pigs submitted to crescent inclusion levels of phosphatidylcholine herbal product during the last 28 days pre-slaughter.

Dose	IBW	FI	ADG	FCR	FBW
0	82,34	2,88	1,01	2,83	112,9
250 mg	82,34	2,87	1,04	2,74	113,8
500 mg	82,34	2,88	1,03	2,79	113,2
750 mg	82,35	2,89	1,04	2,84	113,2
C.V. (%)	1,15	7,31	8,44	6,49	2,42
P Linear	-	0,95	0,88	0,87	0,96
P Reg[2]	-	0,95	0,52	0,41	0,87
PDunnet	-	1,00	0,90	0,79	0,99

For carcass, reduced fat thickness reduction (P <0,10), increased loin depth (P <0,01), no percentage of meat in the carcass (P <0,01) and in the carcass (P <0,10) when the level of the phosphatidylcholine product increased during the previous 28 days. In relation to the control group, the level of 500 mg / kg was more effective in reducing the thickness of the bacon, the muscle depth and the percentage of meat in the carcass. The levels of 250 and 750 mg / kg had better result when compared to treatment with control only in the parameter depth of loin (P <0,05) (Table 2).

Table 2. Carcass evaluation of finishing pigs submitted to crescent inclusion levels of an herbal phosphatidylcholine during the last 28 days pre-slaughter.

Dose	CY, %	BT, Mm	LD, mm	MC %	MQ, kg
0	70,7	15,4	57,7	55,2	44,2
250 mg	70,3	14,5	62,8a	56,0	44,6
500 mg	70,8	13,9a	64,0a	57,0a	45,5
750 mg	70,2	14,2	63,4a	56,8	45,2
CV (%)	3,65	23,8	18,5	6,23	8,58
[1]PLinear		0,06	0,01	0,01	0,10
[1]PReg[2]		-	-	-	0,47
[2]Pdun	0,23	0,10	0,01	0,03	-

1 – P Reg according to Orthogonal Polynomial. Regression with regression related to P < 0,05 for 95% of confidence and P < 0,10 for 90% of confidence. 2 – P Dunnet with control related to the dose without Biocholine (0 ppm)

Conclusions and Discussion

For the conditions and nutritional levels of this study, phosphatidylcholine improves the pig carcass. The level of 500 mg / kg was more effective in reducing the thickness of the bacon, in increasing the percentage of meat in the carcass and in the depth of the loin, when compared to the control group. A possible explanation for this results is related with phosphatidylcholine from herbs mode of action over the glucose and fat metabolism in the body, which can interfere stimulating receptors associated with proteins that upregulates fat burn and downregulates the fat deposition, as adiponectin and resistin, respectively, causing the fat reduction in viscera and bacon (2, 3).

Acknowledgments

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PARASITES AND PARASITIC DISEASES

Family properties of pig breeding: research and extension action to control parasites

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Introduction

Brazil has one of the largest pig databases in the world, consisting of animals used mainly for industrial production, in addition to family systems. In this way, the properties, especially the family ones, have diversified systems of creation, being as parasites one of the biggest obstacles in relation to their production. Pig parasites can cause interference in their development determining economic losses to the producer. In addition, some pig parasites have the potential for zoonotic transmission. Thus, this study aimed to analyze the frequency of parasites in biological samples of pigs and their producers in a family type breeding system in Tanguá, RJ, developing extension actions, between 2017 to 2018.

Materials and Methods

In this study, 14 properties and 132 pigs were included, which were submitted to the collection of fecal samples directly from the rectal ampoule. Of these animals, 117 were also subjected to collection of scrapes from the ear. In addition, stool collection kits were distributed to family producers, 36 people being included in this condition. Fecal samples were subjected to the two flotation techniques[1,2], the two sedimentation techniques[3,4] and to direct examination. The material from the pigs' ear pavilion collected were through direct examination and the flotation technique[5]. In each property, seven technical visits were made where biological samples and information were collected from the application of forms, one with questions about the pigs and their creation and the other with socioeconomic and health questions made to people. In addition, extension activities on parasites were developed in the properties.

Results

Positive for gastrointestinal parasites was observed in 88.6% of the pigs. Non-sporulated coccidia oocysts were the most frequently detected parasites in the positive samples (71.8%), followed by *Balantioides coli* (55%), which are evidenced in all age groups of the animals. It was also detected in pig samples strongylids (40.2%), *Strongyloides ransomi* (31.6%), amoebid cysts (18.8%), *Trichuris suis* (14.5%), *Ascaris suum* (10.2%), nematode larvae (8.5%), *Blastocystis* sp. (3.7%) and (3.7%) and *Capillaria* sp. (0.8%). *Sarcoptes scabiei* variety *suis* was found in 76.2% of the samples that detected ectoparasites. It is important to highlight that the high parasitic positivity evidenced in the studied pigs seems to be related to the lack of sanitary management in the raising of these animals, which was evidenced through the information obtained with the forms, as well as the observations from the technical visits. Most of the producers reported that they had watched the pigs itch, that they had already provided some vaccine and worm medicine for the animals. However, most producers did not remember the name of the drug.

In the fecal samples of producers and their family members, evolutionary forms of parasites were detected in 19.4%, with *Entamoeba coli* (11.1%), *Ascaris lumbricoides* (8.3%) and *Trichuris trichiura* (5.5%) being identified . This series seems to be linked to the city's basic sanitation problems, associated with people's hygienic-sanitary habits, which were evidenced in the form. Extension activities were carried out on family farms to spread information about the parasites of pigs and their producers with a focus on prophylactic measures for their control, called: “interactive lecture, what are parasites and the importance of their control”, “diagnosis walking by property”,“field day”and“ correction of homework ”.

Conclusions and Discussion

It can be seen that several information mediated for improvements in pig health management were adopted by producers. The whole panorama evidenced by the results of this study, demonstrates the need to carry out a program promoted by public agencies, which supports these small producers, who in the case of Tanguá, RJ use pig farming as a means of subsistence and source of income.

Acknowledgments

Pro-reitoria de extensão – Proex/UFF.

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Maintenance of fenbendazole concentrations post oral administration and clinical effect of Pigfen® 40 mg/g premix in pigs naturally infected with *Ascaris Suum*

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Introduction

As well documented in scientific literature, fenbendazole (FBZL) has been evaluated for the treatment of gastrointestinal nematodosis in animals[3]. Despite poor absorption from the gastrointestinal tract, oral administration of FBZL has resulted in clinical efficacy in the treatment of ascaridosis in pigs, which is most probably due to an induced local effect. The objective of this study was to measure the effect on the maintenance of concentrations in the gastrointestinal tract after treatment and to assess the clinical effect of Pigfen® 40 mg/g premix used in pigs naturally infected with *Ascaris suum.*

Materials and Methods

Six groups of 6 pigs (Danube White), of both sexes (28.0-31.5 kg), at about 12 weeks of age, were used. All pigs were naturally infected with *A. suum*. On day 0 of the trial, the infected groups (II-VI) were treated in feed with a single oral dose of 5 mg/kg bw FBZL as Pigfen® 40 mg/g premix. The concentration of FBZL was assessed applying HPLC determination in plasma and small intestinal contents of pigs. Faecal samples were collected and nematode egg counts were determined using a McMaster technique. Efficacy of the medication was evaluated after euthanasia and necropsy of all pigs and the count of adult forms of *A. suum* was determined in the small intestines. The results of the examination were determined according to t-test of Student-Fisher (mean±SEM).

Results

The concentrations of FBZL in the plasma and in the small intestinal contents on the 12th hour after treatment were 0.471±0.04 µg/mL and 2.5±0.12 µg/g, respectively (Table 1). Twenty four and 36 hours after treatment, FBZL concentrations in the plasma were lower as compared to those found on the 12th hour after treatment. Concentrations of FBZL were found in the small intestines contents from the 12th hour to the 72th hour after treatment, the higher concentration of 106.4±4.82 µg/g being found on the 24th hour after treatment.

No statistically significant difference was found in the *A. suum* egg counts in the faecal samples of the pigs of all groups before treatment (Table 2). When included in the feed of infected pigs (II-VI) at a dose of 5 mg/kg bw, FBZL leaded to statistically significant decrease in the *A. suum* worm counts in the small intestines. Worm Count Reduction was 44.97% on the 12th hour and 100% from the 24th hour to the 72th hour after treatment.

Table 1.

Fenbendazole concentrations				
Hours after treatment	Groups	Plasma, µg/mL	Small intestines, µg/g	Small intestines/ Plasma ratio
12 h	I	-	-	-
12 h	II	0.471±0.04	2.5±0.12	5.3
24 h	III	0.307±0.02	106.4±4.82	346.6
36 h	IV	0.092±0.005	62.1±2.99	675.0
48 h	V	<0.005	7.6±0.44	1520.0
72h	VI	<0.005	2.4±0.14	480.0

LOQ <0.005 µg/mL (plasma), <0.5 µg/g (small intestinal contents)

Table 2.

Parameters				
Groups	Average egg count per g of faeces before treatment	Hours after treatment	*A. suum* worm count*	Worm Count Reduction, %
I	95.2±1,57[a]	12 h	2.6	-
II	92.0±1.81[a]	12 h	1.4	44.97
III	91.2±1.47[a]	24 h	0.0	100
IV	94.7±1.59[a]	36 h	0.0	100
V	95.0±1.55[a]	48 h	0.0	100
VI	92.2±1.47[a]	72 h	0.0	100

a p≤0.05; * geometric mean

Conclusions and Discussion

The study results show that in-feed administration of FBZL at 5 mg/kg bw has significant therapeutic effects in pigs naturally infected with *A. suum*. FBZL concentrates in the small intestines and has a high intestines/plasma ratio, which may have a significant role in establishing PK/PD relationships and clinical efficacy against ascaridosis in pigs[2,4]. The present study highlights the maintenance of FBZL concentrations in the intestines and the impact of this reservoir on the evolution of FBZL, which has been demonstrated by statistically significant FBZL concentrations found in the small intestines up to 24 hours after treatment as compared to those found on the 12th, 36th, and 48th hours. The 100% efficacy of FBZL against *A. suum* has also been confirmed in this study[1].

The results obtained indicate that the maintained concentrations of FBZL can help in designing of efficient medication strategies in sustainable pig farming, especially to treat and overcome ascaridosis.

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Ascaris suum antibody detection to guide deworming on pig farms

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Introduction

Traditional detection methods of Ascaris suum on pig farms include: egg detection, worm detection and liver white spot detection. The detection of antibody can be used to detect the Ascaris lumbricoides infection pressure of pigs in all stages.

Materials and Methods

An Ascaris suum antibody detection kit SVANOVIR@ A. suum Ab developed by Ghent University, is based on hemoglobin present in all stages of Ascaris suum development. The infection pressure at the growth stage of pigs in each group was judged according to the average ODR value of 10 samples in each group（Table1）.

We chose a one site farm raising nursery swine on raised plastic flooring and finishers on concrete flooring, hence excluding possible infection in the nursery, and collected sera from 2 to 22-week-old pigs for antibody detection. (Figure1)

We also selected two pig farms with milk spot livers in slaughter check, the nursery pigs of farm 1 were raised on the ground, and the nursery pigs of farm 2 was raised in nursery bed. At the same time, sera of 8, 12, 16 and 20 weeks old were collected for antibody detection.

Result

The antibody test results of the two pig farms were shown in figure 2. The ODR value of pig farm 1 had higher infection pressure at the age of 8 weeks, while in pig farm 2, it showed higher infection pressure after fattening. The result indicated that the infection rules of Ascaris lumbricoides were different under different breeding conditions.

Conclusions and Discussion

Based on our survey, sows are infected with Ascaris suum in sows and piglets have very high maternal antibodies. Antibodies trend downwards before 8 weeks of age, suggesting that the flooring design of the nursery reduces transmission. Based on the serology, the peak burden of Ascaris suum is centred in the finishing barn and treatment to swine in this area should be focused.,

Ascaris suum will continue transmisison amongst swine as long as the faecal-oral transmission cycle is maintained and the result of pig farm 1 indicated the conclusion. Based on these results, we suggest that deworming schemes can be adjusted based on using serological results to assess the timing of infection, followed by gross lesion assessment at slaughter to assess the success of deworming programs.

Table 1

Mean ODR for 10 representative samples	Interpretation
< 0.4	No or low exposure to A. suum
0.4- 0.6	Possibly exposed to A. suum, but limited impact expected
> 0.6	Exposed to A. suum, significant impact expected

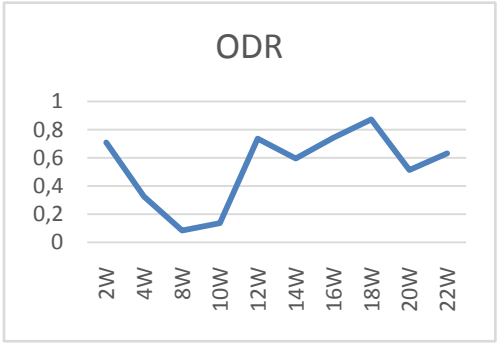

Figure 1

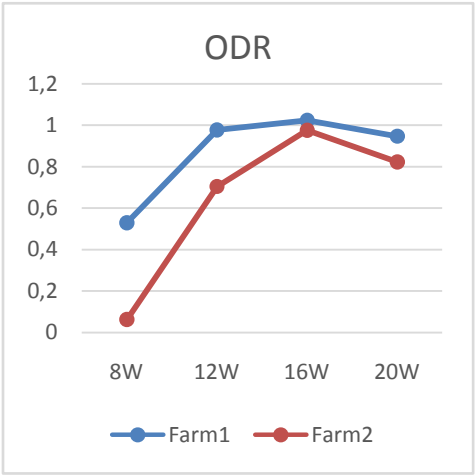

Figure 2

References

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The fecal microbiota evolves differently in piglets experimentally infected with *Cystoisospora suis* that are treated with toltrazuril

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Introduction

The protozoan parasite *Cystoisospora suis* causes diarrhea and reduced weight gain in suckling piglets. Infections with *C. suis* occur in the first days of life; it is transient but can lead to dysbiosis, exacerbating disease and increasing mortality. Cystoisosporosis is effectively controlled by toltrazuril treatment (1); however, alterations of the gut microbial composition upon infection and treatment have not been investigated. This study evaluated the development of fecal microbiota of *C. suis* infected piglets in response to treatment with toltrazuril.

Materials and Methods

Thirty-eight conventional piglets were infected with *C. suis* on the first day of life (dol 1). Twenty-six of them received either parenteral or oral toltrazuril two days later. Fecal samples were collected pre- and post-weaning (dol 1-15 and 31-38) for microbiota analysis using 16S rRNA amplicon sequencing and during dol 5-18 to determine fecal consistency and parasite excretion.

Results

All piglets in the control group shed parasites at least once and the majority developed diarrhea, while toltrazuril-treated piglets did not excrete parasites and only had low levels of diarrhea. Age-related shifts in the fecal microbiota composition and increase in diversity and species richness were seen until after weaning. Infection with *C. suis* disrupted bacterial maturation two weeks after infection (Fig. 1). Irrespective of the route of administration, fecal communities of piglets in the treated groups clustered separately and were more diverse compared to that of control piglets during the acute phase of infection on dol 11. Control piglet feces showed higher levels of *Fusobacteriaceae* and *Veillonellaceae*, while *Ruminococcaceae*, *Lachnospiraceae*, *S24-7*, *Clostridiaceae* and *Erysipelotrichaceae* were more abundant in feces of treated piglets on dol 11. After weaning, treatment-related effects on the microbial communities diminished and were presumably masked by changes associated with weaning.

Conclusions and Discussion

Irrespective of the administration route, toltrazuril prevented *C. suis*-related dysbiosis and maintained species richness and diversity on dol 11. In addition to cystoisosporosis prevention, toltrazuril seems to contribute to the stabilization of the gut microbial development during the suckling phase and thus may reduce the need for antibiotics to control infections with secondary bacterial enteropathogens in *C. suis*-infected suckling piglets.

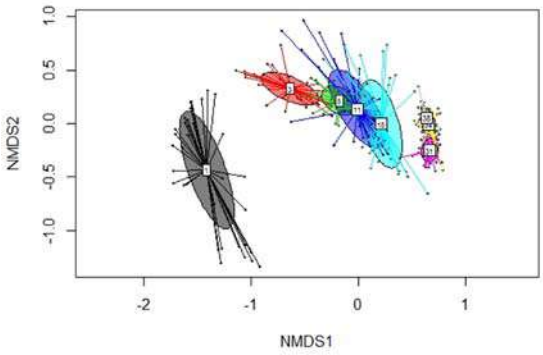

Figure 1. Nonmetric multidimensional scaling (NMDS) plot of pairwise Bray-Curtis dissimilarities among bacterial communities over time at days of life: 1, 3, 5, 11, 15, 31, 34 and 38.

References

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Diagnosis of *Cystoisospora suis* oocysts in piglet samples

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Introduction

Detection of oocysts is a hallmark of the diagnosis of coccidiosis, including suckling piglet cystoisosporosis caused by *Cystoisospora suis*. However, in practice reliable detection is often hampered by the short excretion time of individual animals and the high fat content of suckling piglet samples. In steatorrheic samples the formation of lipid bubbles can lead to misdiagnosis of oocysts (false positive results) and centrifugation leads to formation of fat plugs that can entrap oocysts and completely prevent their recovery from the suspension (false negative results). Repeated sampling of several animals from one litter and several litters from a farm is recommended but the benefit of pooled sample versus single sample examination is poorly examined.

Materials and Methods

Several options for parasite detection in piglet faeces were investigated and compared for speed and ease as well as sensitivity. Fecal samples from experimentally infected piglets were examined by a modified McMaster technique using sugar+staturated sodium chloride solution (1) (lower detection limit: 100 oocysts per gram of feces; opg), staining of fecal smears with 1% carbolfuchsin (2;3) and autofluorescence microscopy (4).

To investigate the effect of repeated litter sampling vs. single samples data from experimental infections were used to create a model to simulate sampling strategies (5). The repeated sampling of litters was evaluated in a field study where samples were taken from litters (several samples from the floor were pooled/litter) in the second and third week of life and the results were compared.

Results

Of the fecal samples (n=30) 21 contained countable oocyst numbers (median: 1665 opg). Autofluorescence microscopy detected oocysts in all 30 samples while only 10 were positive after carbolfuchsin staining (Fig. 1).

The model showed that repeated sampling one week apart returned almost twice as many positive samples compared to single samples (Fig. 2).

Of 180 sampled litters, 70 (38.9%) were positive for *C. suis* at least once, 34 (18.9%) at the first and 53 (29.4%) at the second sampling.

Conclusions and Discussion

Since oocyst excretion by *C. suis*-infected piglets is usually short the right timing and a sufficiently sensitive detection method are important for correct diagnosis. Oocyst detection in faecal smears of samples taken repeatedly is the method of choice to determine extent and intensity of infection on a farm, and autofluorescence microscopy provides by far the lowest detection limit. Other methods for oocyst detection in faeces are less sensitive and/or more labour- and cost intensive and their usefulness is restricted to specific applications.

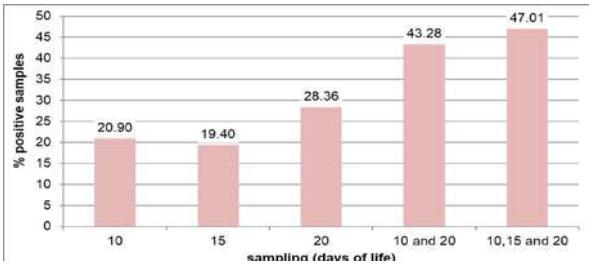

Figure 1. Percentage of positive samples taken on different days of life in a model for different excretion patterns for *C. suis* oocysts.

The most sensitive detection method for *C. suis* oocysts in piglet feces is autofluorescence. Repeated sampling of litters increases the detection rate of oocysts in samples. It is therefore recommended to use autofluorescence for detection and to sample litters/farms repeatedly to return correct diagnostic outcomes for piglet coccidiosis detection and control.

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Efficacy of an injectable toltrazuril – gleptoferron (Forceris®) to control coccidiosis (*Cystoisospora suis*) in comparison with iron supplemented piglets without anticoccidial treatment

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Introduction

Neonatal coccidiosis caused by *Cystoisospora suis* occurs in association with pig husbandry worldwide.[1] Clinical coccidiosis is characterized by yellowish, mostly pasty faeces, reduced body weight gains and weight loss.
Recently a first injectable combination product, Forceris® (30 mg toltrazuril/ml; 133.4 mg iron/ml as gleptoferron - CEVA) has been developed for the control of piglet coccidiosis and the prevention of iron deficiency anaemia. The aim of the multicentric, randomised, blinded study was to evaluate the efficacy of Forceris® against *C. suis* in piglets under field conditions on farms with confirmed coccidiosis.

Materials and Methods

Facts & Figures:

- 1138 piglets naturally exposed were used in the study
- 5 farms (France, Germany, Spain)
- Forceris® group: 45 mg toltrazuril + 200 mg iron i.m. per piglet at either day 1, 2 or 3 of age
- Control group (CP): 200 mg iron i.m. per piglet at day 1
- Faecal examination by modified McMaster method
- Faecal score: 1= firm; 2= pasty; 3= semi-liquid; 4= liquid, diarrhoea defined as Faecal score > 2

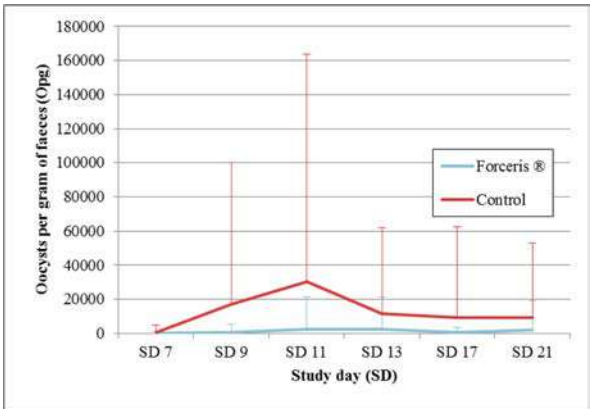
Results

Body weight:

Mean daily body weight gain was significantly higher in the Forceris® group for the period Study day (SD) 0 to SD 14 (p=0.034) and SD 0 to SD 21 (p=0.004) compared to CP group (5936.9 ± 1821.1 g vs. 5625.6 ± 1660.3 g).

Oocyst excretion:

Oocyst counts were consistently higher in the CP group with a peak mean value of 30403.7 Opg at SD 11. In the Forceris® group peak of mean oocyst excretion was reached two days later and was significantly lower with 2506.2 Opg. The area under the curve of oocyst excretion was significantly lower in the Forceris® group ($p < 0.001$).



The number of faecal samples with positive oocyst count was reduced in piglets treated with Forceris®.

Diarrhoea:

The success of Forceris® treatment regarding the control of diarrhoea was clearly demonstrated in all farms (22.7% of the piglets presenting diarrhoea at least once in the Forceris® group versus 44.4% in group CP, $p < 0.001$).

Conclusions and Discussion

The results clearly demonstrated the efficacy of Forceris® at a fixed dose of 1.5 ml/piglet (45 mg toltrazuril plus 200 mg iron) when administered once at either day 1, 2, or 3 of age. Coccidiosis due to *C. suis* was successfully controlled as Forceris® treated piglets displayed significantly reduced oocyst excretion, less diarrhoea and improved body weight gain.

References

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Early application of parenteral toltrazuril-iron combination (Forceris®) can control experimental cystoisosporosis in suckling piglets

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Introduction

Cystoisosporosis (coccidiosis) is a leading cause of diarrhea in suckling piglets and is controlled by metaphylactic toltrazuril application (1). Recently, a single dose combination product (Forceris®) has been developed for the prevention of piglet cystoisosporosis and iron deficiency anaemia. It is applied intramuscularly between the 1st and 3rd day of life (day of life=study day; SD) (24-96h after birth). In previous experimental studies, it was shown that treatment with Forceris® on the 2nd study day (SD followed by experimental infection with *Cystoisospora suis* on the 3rd SD eliminated the oocyst shedding and reduced diarrhoea and to consequently improves body weight gain and health of treated piglet compared to infected untreated control (2).

Materials and Methods

A subsequent study with experimental infection conducted on the 1st SD and treatment on the 2nd SD was conducted to determine the efficacy of Forceris® when applied after the onset of neonatal infections. Piglets were randomly assigned to the Forceris® group (n=13; 45 mg toltrazuril + 200 mg iron/piglet), and to the Control group (n=12; 200 mg iron/piglet). General animal health was recorded daily, and body weight was determined weekly during the study (1st – 29th SD). Individual faecal samples were collected from the 5th - 18th SD and examined for faecal consistency and the presence of oocysts.

Results

In the Control group all piglets shed countable oocysts, while the Forceris® group remained negative ($p<0.0001$) (Fig. 1). Diarrhoea was seen in all animals in the Control group and in one animal in the Forceris® group ($p<0.001$) (Fig. 1). The body weight gain was significantly depressed in the Control group compared to the Forceris® group during the first two weeks post-challenge ($p=<0.0001$) (Fig. 2).

Conclusions and Discussion

Forceris® was safe to use and effective in a single application against experimental infections with *C. suis* on the 1st dol and can be recommended for treatment of porcine coccidiosis in neonatal piglets (3).

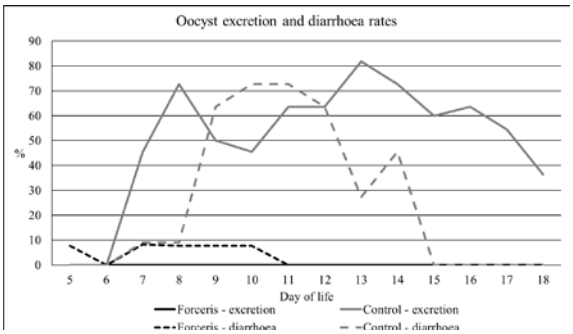

Figure 1. Oocyst excretion rates (%) and percentage of diarrheic piglets in the two groups (Forceris: n= 13, Control: n=11) from the 5th to the 18th day of life. Infection: 1st day of life, treatment with Forceris: 2nd day of life.

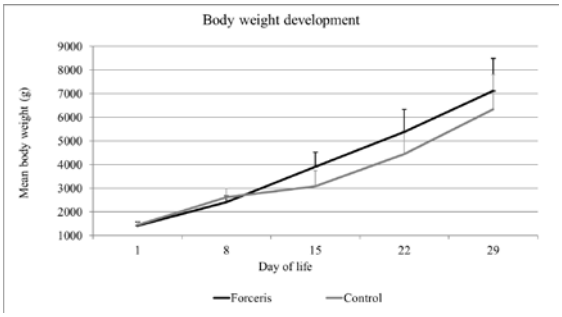

Figure 2. Body weight development in the three groups. Details: see Fig. 1.

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PRODUCTION & INNOVATION

Efficacy of needle-free iron injection, an on farm double blind randomized controlled clinical trial

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Introduction

Pigs are born with a low reserve of iron (±50 mg) and the sow's milk contains insufficient amounts of iron (±0.2 mg/100 ml) to meet the iron needs of the piglet (±10 mg/day). Pigs with insufficient iron supply will develop a hypochromic, microcytic anaemia. Recent literature suggests that piglets with iron deficiency not only develop an anaemia but are also considered to become cognitially impaired, for instance spatial cognition (1,2). Therefore, iron supplementation seems to remain relevant for pig health and welfare. Currently, in pig husbandry, piglets receive an intramuscular injection with 200 mg iron around three days of age for the prevention of anaemia. In recent years an increased interest is noted for needle-free injections, because of efficiency as well as safety for man and animal.

This study aims to support the evidence on efficacy of needle free iron delivery. To this aim the study has two objectives:

1) To determine the efficacy of needle-free iron delivery to prevent iron deficiency anaemia in pigs at weaning (day 26)

2) To compare the efficacy of needle-free iron delivery with conventional injection by needle.

Materials and Methods

A double blind randomized clinical trial was conducted with 72 pigs from 9 litters, according to Kievit (3). From each litter 3 piglets were randomly allocated to the needle group, 3 to the needle-free group and 2 pigs to a non-treated control group. Pigs were weighed and blood (EDTA + Serum) was collected at D3, D14, D26 and D70. It is known that after birth Haemoglobin (Hb) and serum iron (Fe) slowly decline and that uptake of iron by the piglet is insufficient to warrant sufficient production of Hb. Individual variation of fetal Hb-degradation and iron uptake from the environment can result in considerable individual variation in blood parameter values, especially in the control group. Therefore, it is needed to obtain insights in the starting point (day 3), de decline (day 14 and/or day 26) and increase (day 26 and 40) in individuals. The laboratory analyses consist of Hb, Haematocrit (Ht), differentiated red blood cell parameters, serum iron, iron binding capacity en iron saturation.

A linear mixed effects model with random litter effect was used to compare the effect of needle-free injection on serum haemoglobin and haematocrit respectively on D26.

Results

Although variation of Hb on D26 was increased in the needle-free group compared to the needle group, no significant differences between needle-free and regular injection of iron on Hb and Ht at D26 were found. In the control group, however, Hb and Ht at d26 was significantly lower. Interestingly, weight of the pigs between groups did not differ at any time point, in contrast to literature (4). Other haematological results showed almost equal results for both needle-free and needle injection group. No adverse signs were noted in all groups.

Conclusions and Discussion

Needle-free iron injection is as effective and seems as safe as injection per needle to prevent anaemia at weaning.

Table 1. Descriptive results of mean Haemoglobin (Hb), Haematocrit (Ht) and Body weight (Kg) on day 26. In addition 95% confidence intervals are obtained by univariate t-tests of the parameters for that specific group at that specific point in time (Day 26).

Study group	*Hb on D26 (95% CI) (mmol/L)*	*Ht on D26 (95% CI) (%)*	*Body weight on D26 (95% CI) (Kg)*
Control (C)	5.9 (5.4 – 6.7)	0.32 (0.30 - 0.34)	7.9 (7.1 – 8.6)
Needle (N)	7.3 (7.2 – 7.5)	0.38 (0.37 – 0.39)	7.9 (7.4 – 8.4)
Needle-free (NF)	7.3 (7.0 – 7.6)	0.37 (0.36 – 0.39)	7.5 (7.0 – 8.1)

Acknowledgments

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Use of skin infrared thermography to detect hypothermic piglets in farrowing room

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Introduction

The newborn piglet has thermal critical needs during the first hours of life to survive, based on heat and colostrum intake. If the piglets fail finding a heat source or eating immediately after birth, or if we fail providing such source or attending the farrows, the piglet can become hypothermic and finally dye.

This work was done to validate a smart thermal camera and an App to assess body temperature on the basis of infrared skin emission assessment, and to establish parameters to decide when it's necessary to act on the piglet or when our efforts will not be useful to save his life.

Materials and Methods

One hundred and fifty-eight piglets were used in this investigation, Duroc x (Landrace x Large White), from a commercial farm in South-East Spain. The farrow boxes were provided with a heat source (IR lamp) and farrows were attended by qualified staff. Thermographies (Tir) were done by means of a FLIR ONE Pro for Android (Flir Inc, USA) thermalcamera accoplated to a smartphone, and using the App Degree2act (Beinfive, Spain). The piglets were weighted with an electronic scale and body temperature was assessed with an electronic clinical thermometer (Trec).

Thermographies and body thermometric temperature were assessed at 12 and 36 hours after birth. The animals were selected on the basis of a behavior compatible with hypothermia, and a littermate showing normal behavior was also investigated at same moment.

Results

Finally, 291 thermographies were performed on the piglets, 158 at 12 hours of life and 133 at 36 hours after birth. The correlation between Trec at 12 and 36 hours was r=0.705 (p<0.001) and correlation between Tir at both moments was also significant (r=0.0338, p<0.001). The correlation between Trec and Tir was r=0.511 (p<0.001) and r=0.423 (p<0.001) at 12 and 36 hours after birth.

There also was a correlation between weight and temperatures. The correlation at 12 hours between weight and Trec was r=0.684 (p<0.001) and r=0.572 (p<0.001) at 12 and 36 hours, respectively. And with Tir was r=0.289 (p<0.001) and r=0.322 (p<0.001) at both times.

As regards the relation between weight, temperature and mortality, both parameters showed influence on mortality during first 36 hours of life. The average weight, Trec and Tir for animals surviving or not at 36 hours are shown in Figure 1. There was significant differences for all the three parameters (p=0.008, p=0.001, p<0.001 for weight, Tir and Trec, respectively). There was no influence of sow parity on the results.

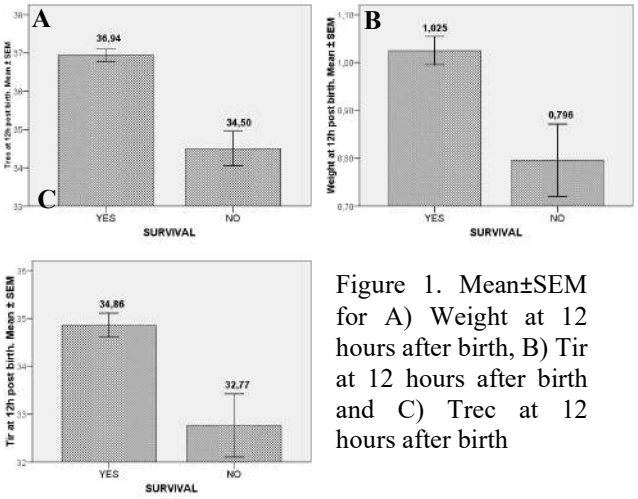

Figure 1. Mean±SEM for A) Weight at 12 hours after birth, B) Tir at 12 hours after birth and C) Trec at 12 hours after birth

Conclusions and Discussion

The weight at birth is one of the most important factors for survival of piglets[1,2]. The animals which didn't survive, had a significant lower mean temperature at 12 hours after birth. In fact, 65% of them were under 35ºC. Is well known that the size of the animal (weight) is related with the capacity to preseve body heat[3], with small piglets lossing very quick temperature after birth. In fact, all animals, except for two, dying before 36 hours of life weighted less than 700 g at birth. The use of non-invasive tools such as thermography allow us to detect hypothermic piglets and to decide if they need for heat suplementation or otherwise there is no chance of survival and we should not invest time or effort with them. The correlation of Trec and Tir indicates that the tools is useful for the purpouse.

In conclusion, IR thermography, combined with weight assessment and behaviour evaluation, will help to manage small hypothermic piglets avoiding use invasive technics with time saving, non iatrogenic transmission by vehicles such as thermometers, and allowing a quick intervention on these piglets.

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Effects of Improvac® vaccination in female pigs intended for market versus intact females: Meta-analysis of most relevant parameters for pig producers

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Introduction

Improvac®, an anti-GnRF (gonadotropin-releasing factor) vaccine has recently been licensed in Canada, Central and South American countries for temporary suppression of ovarian function and the suppression of estrus in intact female pigs intended for slaughter. Because of the immunization, gilts also increase their harvest weights with a dosing scheme like the male pigs[1]. It results in higher average daily gain while suppresses behavioral injuries such as mounting and aggression[2], besides diminishing the risk of unwanted pregnancies when gilts are raised with uncastrated boars in some countries and farms. The aim of the current meta-analysis study is to analyze the effect of the immunization with Improvac® in female pigs intended for market compared to untreated gilts in presence of signs of estrus and how this affects the growth performance parameters that are most relevant for pig producers.

Materials and Methods

A comprehensive database served as source of studies on the effects of Improvac® vaccination to female pigs intended for slaughter, briefly known as the market gilts; summing up to 38 studies at the time of data search. The inclusion criteria were data from published studies estimating the vaccination effect of Improvac® in gilts on percentage of estrus detection, average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), final live weight (LW) and backfat (BF) compared to untreated gilts; while data of the published studies using the feed additive ractopamine were excluded. Meta-analysis techniques were used to estimate the mean differences between vaccinated versus the untreated market gilts using the statistical software Comprehensive Meta-Analysis V.2.2 (Biostat, Englewood, NJ). For each parameter Z statistic and corresponding P-value were used to determine if differences between immunized gilts against GnRF and untreated gilts were statistically significant[3]. The I^2 statistic was used to describe the percentage of total variation across studies which cannot be explained by chance[4]. For the detection of signs of estrus, studies using an observation period ≤ 24 hours were excluded as it was reported to be insufficient to detect its signs in vaccinated or untreated gilts[1].

Results

A total of 22 scientific articles fulfilled the defined inclusion criteria. Results of parameters defined as most relevant to pig producers are presented in Table 1 (Estrus detection, live production or growth performance and final harvest measurements including comparisons without ractopamine feed additive). The growth parameters (ADG, ADFI, FCR and Final LW) were recorded presumably the overall period of observation in the studies over post-second dose vaccination period to the harvest day of the gilts.

Table 1. Results of meta-analyses on estrus and most relevant parameters for pig producers.

	N	RR	P-value	I^2
Estrus detection (%)	4	0.044	<0.001	46.8%
	N	RMD	*P*-value	I^2
ADFI (kg/day)	8	0.190	<0.001	79.3%
ADG (g/day)	11	45.11	<0.001	0.0%
FCR (kg/kg)	6	0.030	0.556	81.8%
Final live weight (kg)	11	4.000	<0.001	11.9%
Backfat (mm)	11	2.825	<0.001	20.6%

n = number of comparisons; RR = risk ratio; RMD = raw mean difference

Conclusions and Discussion

The probability of estrus signs detection was reduced by >95% in vaccinated gitls compared to untreated gitls (RR=0.044; P<0.001), using an observation period of ≥ 17 days in all studies. ADFI was higher in vaccinated gilts compared to untreated gilts (0.19 kg/day; P<0.001). ADG was on average 45.1g/day higher in vaccinated gilts vs untreated gilts (P<0.001) while the FCR was similar between them (P=0.556). Final LW was on average 4.0 kg higher in Improvac® vaccinated gilts compared to untreated gilts (P<0.001) while backfat was on average 2.8 mm thicker in vaccinated gilts (P<0.001).

Based on our meta-analyses results, vaccination with Improvac® effectively reduces the occurence of estrus signs in females pigs intended for market. It also results in additional benefits for pig producers that can raise finishing female pigs with a better ADG and heavier final LW, and with no negative impact in FCR, as the increase in feed intake after vaccination is compensated with the additional growth.

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A comparison of immunizing female pigs intended for market against GnRF versus untreated gilts: meta-analysis of parameters relevant for pork packers

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Introduction

The vaccine against gonadotropin releasing factor (GnRF) Improvac© (Zoetis) has recently emerged as a viable option for inducing temporary suppression of ovarian function and the suppression of estrus in intact female pigs intended for slaughter. This also result in gilts achieving better average daily weight gain (ADWG) and heavier harvest weights as non-cycling females have a higher and more consistent feed intake during the late finishing phase[1, 2]. The aim of our study was to use meta-analytic techniques to assess the magnitude of the effect of immunized gilts compared to untreated gilts based on those parameters that are most relevant for the pork packing industry, i.e. carcass quality, including the overall gain of valuable meat, as well as the effect in reproductive organs (ovarian and uterine weights).

Materials and Methods

A contemporary electronic database containing 38 publications reporting the use of anti-GnRF vaccine, Improvac© in female pigs were used to identify those eligible that included parameters of interest to pork packers. Meta-analyses were conducted using the statistical software Comprehensive Meta-Analysis V.2.2 (Biostat, Englewood, NJ). For each parameter Z statistic and corresponding P-value were used to determine if differences between immunized gilts against GnRF and untreated gilts were statistically significant[3]. The I^2 statistic was used to describe the percentage of total variation across studies which cannot be explained by chance[4].

Results

A total of 22 papers fulfilled the defined inclusion criteria. Results of parameters defined as most relevant to pork packers are presented in Table 1 (General carcass traits) and Table 2 (Yield of valuable meat and weight of reproductive organs).

Table 1. Results of meta-analyses of general carcass traits defined as most relevant for pork packers.

	N	RMD	*P*-value	I^2
Carcass weight (kg)	10	3.240	<0.001	28.2%
Dressing (%)	8	0.213	0.220	22.0%
Muscle depth (mm)	4	-0.081	0.484	0.0%
Percentage lean (%)	7	-1.521	<0.001	0.0%
Intramuscular fat (%)	6	0.207	0.001	0.0%

n = number of comparisons, RMD = raw mean difference.

Table 2. Results of meta-analyses of yield of valuable meat most relevant for pork packers and weight of reproductive organs.

	n	RMD	*P*-value	I^2
Ham (kg)	5	0.024	0.861	60.1%
Shoulder (kg)	3	-0.057	0.698	37.3%
Loin (kg)	6	-0.062	0.216	80.9%
Belly (kg)	4	0.277	<0.001	0.0%
Ovary (g)	6	-6.960	<0.001	59.3%
Uterus (g)	3	-569.760	<0.001	10.5%

n = number of comparisons, RMD = raw mean difference

Conclusions and Discussion

Compared to untreated gilts, immunized gilts had on average 3.2 kg higher carcass weight (P<0.001), while no statistical difference in dressing percentage (P=0.220) and muscle depth (P=0.484) was found. On average immunized gilts are 1.5% units less lean and have 0.21% units more intramuscular fat than untreated gilts. Yields of ham, shoulder and loin were not signicantly different between immunized and untreated gilts. Yield of belly was on average 0.28 kg heavier in immunized gilts. Reproductive organs were significantly (P<0.001) smaller (7.0 g lower ovarian weight and >0.5 kg lower uterine weight) in immunized gilts.

Based on our analyses, as dressing percentage is smilar between immunized and untreated gilts, the higher final live weight results in higher carcass weight, which leads to higher uniformity within mixed genders batches. Additionally, the lower percentage lean and increased fat content as well as higher belly weight diminish the often critized ”over-leanness”of female pigs intended for markets. Therefore, the vaccination of gilts with Improvac© is a reliable opportunity for pork packers to process pork carcasses with higher value.

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Field efficacy of an injectable toltrazuril and gleptoferron formulation (Baycox® Iron Injection) in comparison to injectable gleptoferron for the prevention of iron deficiency anemia in piglets

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Introduction

Pigs raised in intensive farming conditions are at risk of developing iron deficiency anemia (IDA). Different factors are associated with the occurrence of the disease in piglets, such as low iron reserves at birth, fast growth rate compared to other species and low iron levels in sow's milk (1,2). Recently, a novel, patented combination of toltrazuril and iron (Baycox® Iron Injection) has been developed, combining prevention of IDA and coccidiosis caused by *Cystoisospora suis* (3, 4). This study evaluated the IDA prevention efficacy of Baycox® Iron Injection in commercial farm conditions in comparison to a commercial injectable gleptoferron iron.

Materials and Methods

In this blinded study conducted in commercial farms in Portugal, 223 piglets were randomly allocated into two treatment groups. Group A (n=112) received a 200 mg IM injection of Ursoferran® (gleptoferron 200 mg/mL). Group B (n=111) was treated intramuscularly with Baycox® Iron Injection at the dose of 20 mg/kg BW of toltrazuril and 100 mg/kg BW of iron (as gleptoferron). Treatments were administered on day 3 of life. Piglet body weight (BW) was assessed in all animals to evaluate performance and as a surrogate variable to IDA prevention. Blood samples for hemoglobin (Hb) evaluation were randomly collected from a subset of animals (23 and 18 animals from groups A and B, respectively), on days 3 and 16 of life.

Results

On day 3, BW was 2.102±0.434 and 2.077±0.453 kg, for groups A and B, respectively (p=0.79). Baycox® Iron Injection treated piglets (Group B) showed numerically higher BW on day 16 (4.414±0.906 vs 4.363±0.842 kg, p=0.54), total BW gain from day 3 to 16 (2.336±0.673 vs 2.261±0.607 kg, p=0.45) and daily weight gain (180±52.4 vs 174±46.5g/day, p=0.44). Hemoglobin levels on day 3 were similar in both groups (Group A: 8.29±1.55 g/dL and Group B: 8.66±1.89 g/dL, p=0.61). On day 16, there was no statistical difference on hemoglobin levels between groups (Group A: 11.58±1.43 g/dL and Group B: 12.07±1.24 g/dL, p=0.25).

Conclusions and Discussion

The study showed that the weight dependent iron supplementation dose of 100 mg/kg provided by Baycox® Iron Injection performed at least on the same level as a fixed 200 mg iron injection per animal, being effective in preventing iron deficiency anemia, and ensuring proper weight development of piglets in field conditions.

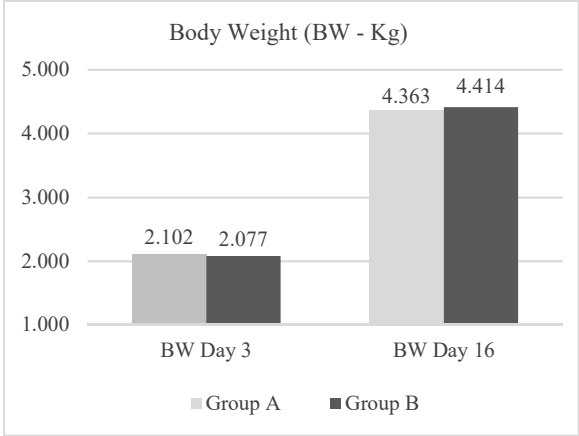

Figure 1. Body weight (BW) on day 3 and day 16 of the study – Group A (Control – 112 piglets) / Group B (Baycox® Iron Injection – 111 piglets).

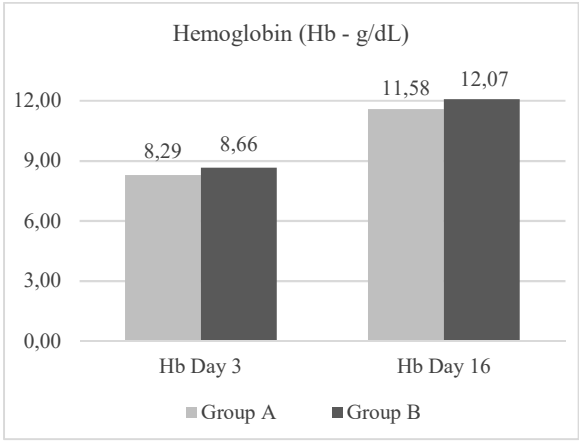

Figure 2. Hemoglobin (Hb) on day 3 and day 16 of the study – Group A (Control – 23 piglets) / Group B (Baycox® Iron Injection – 18 piglets).

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Field applications of population sampling methods to monitor and surveil PRRSV

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the major global swine diseases. It is caused by PRRS virus (PRRSV). Population sampling methods (PSM) including processing fluid[1] (PF) collected at 3 to 5 days post farrowing; family oral fluid[2] (FOF), i.e., the collective OF from the sow and due-to-wean pigs; and oral fluid[3] (OF) to surveil and monitor PRRSV have been developed. The modern technology of PSM help veterinarians to monitor and surveil PRRSV activity in an efficient/precision way, helping to take informed decisions to control or eliminate the virus from pig populations. The purpose of this abstract is to summarize key findings on applicable ways to surveil and monitor PRRSV using PSM.

Materials and Methods

Our group has conducted several field applicable research studies to assess the feasibility and practicability of monitoring PRRSV in breeding herds and growing animals using PSM, i.e., PF, FOF, and OF. In this summary we describe the major findings, highlighting the field applications of such tools.

Results

PF was first reported in 2017[4] and OF in 2008[3]. Based on the Swine Disease Reporting System information, during 2019 PF and OF combined represented 51% (37,138 of 72,865), of submissions tested for PRRSV RNA by RT-PCR in 4 major swine-centric veterinary diagnostic laboratories in the USA.

PF-based sampling is a practical way to monitor PRRSV RNA or antibodies in 3-5 days old piglets during castration and tail docking time[1]. When there was a single viremic piglet in a room there was a 90%, 80%, 60%, probability to detect PRRSV by qPCR when pooling PF from 35, 45 and 58 litters respectively[5].

PF samples have been used to screen PRRSV activity in breeding herds undergoing virus elimination[6]. The median time to stability was 27 weeks (range 18-55 weeks). After 8 consecutive weeks of PCR-negative results in PF, 60 due-to-wean piglets sera were tested for PRRSV. When PCR-negative results were obtained gilts were re-introduced in the farm. Using this protocol, 93.75% of herds confirmed to be PRRSV-stable after 3 months post gilt introduction, high from 87.5%[7] success rate previously reported in the literature when only 30 weaner pigs/month were tested, allowing gilts in after 4 consecutive PCR-negative tests.

Testing 30 FOF from due to wean piglets allows detecting PRRSV RNA at 1% prevalence, which is equivalent to testing 298 individual due-to-wean sera samples. FOF were able to detect PRRSV after PF samples tested negative up to 11 consecutive weeks[8]. Since PRRSV can sustain infection at very low prevalence, when a herd tests PCR-negative on PF this may not correspond to negative PCR-results of weaning pigs. Thus, once there are 6-8 consecutive PCR-negative results in PF, it is recommended testing the due-to-wean pigs by FOF or sera, to confirm lack of PRRSV detection, especially for herds undergoing PRRSV elimination.

PRRSV RNA RT-qPCR Ct values obtained on PF were used to classify weekly batches of newborn piglets according to 4 PRRSV exposure groups (EXPG): 'low Ct' when $Ct \leq 27$, 'medium Ct' when $27 < Ct < 34$, 'high Ct' when $34 \leq Ct < 37$, and 'negative PCR' when $Ct \geq 37$. The nursery closeout mortality was associated with EXPG. EXPG 'low Ct' had 3.51 (range 3.28-3.99) percentage points higher mortality than other PRRSV EXPG. Classifying weekly batches according to PRRSV status demonstrated to be a great predictor of nursery mortality and allows to develop strategies to minimize the impact of the virus in growing animals.

Monitoring PRRSV in wean-to-finish groups by collecting 6 OF every 3 weeks, spatially distributed in the barn, starting at week 4 weeks of age up to 25 weeks was able to detect 3 different patterns of infection: early-infection (EI), mid-infection (MI), late-infection, and no-infection (NI)[9]. Groups having EI and MI had significant higher growing mortality ($p<0.001$) when compared with the NI. This work suggests the earlier the infection with PRRSV in the growing phase, the higher is the expected the wean-to-finish mortality to be.

Conclusions and Discussion

PSM are easier to collect, more practical, and offer better herd sensitivity to monitor PRRSV than bleeding 30 or 60 pigs. The fact that PRRSV can sustain infection in a population at very low prevalence demonstrates the need to monitor as many piglets, crates, rooms as possible over time to increase the confidence that PRRSV is not circulating before re-introducing naïve gilts. PF and OF RT-qPCR test results demonstrated to be a useful tool to classify batches according to PRRSV status and to provide useful information for taking informed decisions to develop intervention strategies to improve pig health, welfare, and productivity.

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Field and research applications for pathogen monitoring in the United States by PCR at veterinary diagnostic laboratories

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Introduction

The Swine Disease Reporting System (SDRS) aggregates information derived from nucleic acid detection by PCR-based assays from 4 major swine-centric veterinary diagnostic laboratories (VDLs) in the United States (US)[1,2], representing 95% of the porcine testing performed in the US-accredited USDA National Animal Health Laboratory Network (NAHLN). Near real-time information is reported for porcine reproductive respiratory and syndrome virus (PRRSV), porcine epidemic diarrhea virus (PEDV), porcine deltacoronavirus (PDCoV), transmissible gastroenteritis virus (TGEV), and *Mycoplasma hyopneumoniae* (MHP). The objective of this work is to summarize the major findings of the SDRS project, which supports informed animal health decisions by veterinarians and researchers.

Materials and Methods

Clientele anonymized submission information, PCR tests and test results were retrieved from Iowa, Minnesota, Kansas, and South Dakota State University's respective VDLs. Submission level information and data was organized in a standardized format, data from the 4 VDLs were consolidated, and presented on an online platform to summarize findings over time, age category, sample type, and geographical location. Algorithms were developed to scan the historical and current information for emerging or re-emerging trends of detection. A cyclic robust regression model was applied to the historical percentage of positive submissions to forecast the expected upcoming year weekly percentage of positive results, with a 95% confidence interval. Alert signals were considered when the detection crossed the expected confidence boundaries. Major findings and signals were validated by a panel of specialists and compiled in monthly reports during years of 2018-19.

Results

As of December 31st 2019, the number of historical submissions tested and added to the SDRS database was 627,040 for PRRSV, 225,091 for PEDV, 159,998 for PDCoV, 134,235 for TGEV, and 74,674 for MHP. The highest percentage of positive submissions of PRRSV, PEDV, PDCoV, and TGEV consistently occurred during colder months of the year (Dec-May), and for MHP during fall months (Sep-Nov). Detection of TGEV almost disappeared after the introduction of PEDV and PDCoV to the US in 2013-2014. Detection of PRRSV in growing animals precedes the increased detection in breeding herds. During 2019 oral fluids (OF) and processing fluid (PF) consisted of 35.04% (25,529 of 72,865) and 15.39% (11,608 of 72,865) of the submissions tested for PRRSV, respectively, demonstrating the importance of population-based monitoring in contrast to individual specimen types. OF-based submissions also represented 51.22% of cases (18,051 of 35,241) for PEDV, 53.75% (16,891 of 31,426) for PDCoV, 54.38% (16,397 of 30,152) for TGEV, and 33.5% (3,094 of 9,237) for MHP testing. The age categories having highest number of submissions for testing within each agent were: suckling piglets represented 26.26% (19,131 of 72,865) of PRRSV cases, whereas grow-finish cases were 21.41% (7,545 of 35,241) for PEDV, 19.84% (6,234 of 31,426) for PDCoV, 20.12% (6,067 of 30,152) for TGEV, and 36.26% (3,349 of 9,237) for MHP. Cyclic models were able to forecast and predict the expected upcoming year's results. Alert signals were issued at different time points of the years for different agents[3,4]. Dashboards and monthly reports can be accessed at: https://www.fieldepi.org/sdrs.

Conclusions and Discussion

The SDRS project is able to summarize test results on a near real-time basis, and keep the US swine industry informed of the megatrends of agent detection by PCR over time using major US swine-centric VDLs results. This information has been widely used by veterinarians, researchers, and producers to acquire insight for agent detection and monitoring. Benchmarking purposes at a national, regional or state level between the SDRS-information and production system-specific datasets are used to make informed decisions on disease management.

Algorithms were able to scan the summarized information, forecast the upcoming year expected results, and inform US swine stakeholders on unexpected detection on a national level. Alert signals have been used to trigger and reinforce biosecurity compliance for people, visitors, transportation, and animal movement practices as measures to help in decreasing further spread of these agents.

SDRS presented results are informative of agent detection on samples submitted for testing at participant VDLs; however, they and are not sufficient to infer disease occurrences, incidence or prevalence.

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Meta-analysis on 14 studies proves efficacy gut health additive in improving performance

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Introduction

Antimicrobial growth promoters (AGP) are used at low levels for economic reasons, however have the highest risk for inducing antimicrobial resistance. AGPs improve growth performance via direct effects on microbiota in the intestinal tract. Finding new ingredient combinations to support microbiota balance and enhancement of gut integrity can give a broader mode of action and supports gut health and consequently performance. Several products based on gut health improving additives (GHA) are available in the market. However, in practice, the scientific documentation behind the efficacy of various forms of GHA is limited and needs to be experimentally supported to identify the most suitable form optimal for piglet performance. A meta-analysis is a powerful tool that combines the data from multiple comparable studies with increased power and robustness of the analytical method. In the meta-analysis described in this abstract the effects of a synergistic blend of gut health improving additives in weaned piglets from 14 scientific studies have been combined.

Materials and Methods

A meta-analysis was performed on the data from 14 scientific trials conducted in 8 different research institutes globally between 2012 and 2017. A total of 2,668 animals were included in the analysis, where treatments consisted of Negative Control (NC, control diet, no AGP or additives) and PFX (control diet + synergistic blend of organic acids, medium chain fatty acids, patented alkyl esters of MCFA's, target release butyrates and a phenolic compound, 0.2-0.3%). Piglets were either not challenged or exposed to a hygiene challenge. Copper was included at nutritional levels and ZnO was included at nutritional or pharma levels in diets according local circumstances in the specific countries. The study duration ranged from 28 to 42 days. Average weight of the piglets at the start of the trials was 6.35kg and an average final body weight of 19.30kg was reached for the NC and 19.97kg for PFX. Raw data from the individual trials were combined into one dataset and analysed using a random effects mixed model procedure for feed efficiency (FE) and average daily gain (ADG). Economic benefits were calculated based on the average outcome of the analysis.

Results

The inclusion of PFX in piglet diets resulted in an overall average significant improvement of 6.2% ($p < 0.001$) in ADG in piglets with PFX included in the diet compared to negative control (Figure 1). FE was significantly improved by 3.2% on average ($p < 0.001$) for the PFX treatments compared to negative control. Economic calculations showed that PFX investment is € 0.15 per piglet. With the increase in ADG and improvement in FE the PFX investment results in an additional € 1.31 financial gain per piglet, taking in account current market prices (ROI 6.4).

Conclusion and discussion

A previous meta-analysis with more than 1,000 studies between 1950 and 1985 demonstrated that AGPs improved the ADG in weaned piglets by an average of 16.4% and the FE by 6.9% (1). In more recent studies (>1985) it was shown that the growth promoting effects of AGPs in weaned piglets are much less pronounced; 5.0% and 1.4% respectively (1). The current meta-analysis demonstrates that the average improvements of zootechnical performance found with PFX are higher compared to the reported recent efficacy of AGPs.

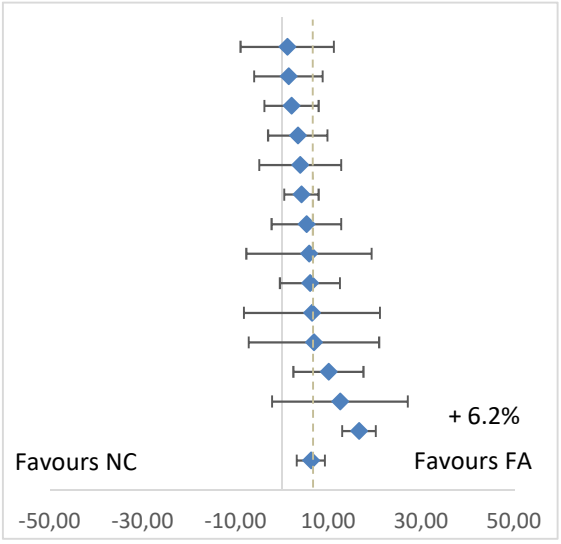

Figure 1. Meta-analysis results on ADG of weaned piglets with or without FA in the diet, indicated in %. Results above 0.00 favours FA treatment and -0.00 favours negative control treatment*

*Data points are differences of means with error bars representing 95% confidence interval. Size of circular data point indicates weighting of trial within the meta-analysis. Diamond data point denotes the overall result of meta-analysis (i.e. mean difference; $p < 0.001$). A result of 0.00 indicates no difference. Dotted vertical line denotes mean difference determined by meta-analysis.

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Single or blended organic acids: Which is more effective for weaned piglets in commercial production?

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Introduction

The use of organic acid (OA) based products to improve the performance of piglets has been reported in several studies (1, 2). Although, the potentiated efficacy of these products varies depending on the forms, types and concentrations of acids (2), nonetheless, evidence exists that OA can be used as a viable replacement for antibiotics to overcome post-weaning growth lag. As observed in experimental conditions, the dietary supplementation of single OA (i.e. citric, formic, butyrate) can increase growth and improve feed efficiency (1); whereas the use of synergistic blend (short– and medium–chain fatty acids) exert a stronger effect on piglet performance and health, due to the multiple functions including broad antimicrobial activity (1).

Nowadays, several products based on a single or blended form of OA are available in the market. However, in practice, the scientific documentation behind the efficacy of various forms of OA is very limited and needs to be experimentally supported to identify the most suitable form optimal for piglet performance. This study was conducted to investigate the efficacy of single or blend of OA on performance and health of weaned piglets under commercial farm conditions.

Materials and Methods

Six hundred weaned piglets with an average initial body weight of 7.5 kg were used in a farm trial in Vietnam. Pigs were distributed to one of five treatments with 12 replicate pens of 10 pigs each according to sex and weight. The treatments included a negative control (NC, an AGP-free basal diet with 2500 ppm ZnO), antibiotic (AGP, as NC + 200 ppm Colistin/T feed), coated butyrate (Butyrate, as NC + 2 kg butyrate/T feed), synergistic blend of free and buffered OA (SPH, as NC + 1.5 L SPH/1000 L water) and a synergistic blend of free and buffered short– combined with medium-chain fatty acids (SGG, as NC + 2.5 kg SGG/T feed). Piglets were fed with a two-phase diet based on corn-soya over a 35-day feeding period. Data on growth performance were analyzed using a GLM in SAS and chi-square was used for diarrhea incidence.

Results and discussion

Irrespective of the sources, the supplementation of single (butyrate) or synergistic blend (SGG & SPH) of OA stimulated ($P<0.05$) ADFI and subsequently improved ($P<0.05$) the ADG and FCR compared to the NC, but not against the AGP treatment (Table 1). Among the additives, SGG and SPH proved to be more effective than butyrate in enhancing the growth performance of piglets. The supplementation of SGG and SPH improved ADG by about 15-16% and reduced FCR by 8-9% compared to butyrate application (Figure 1). All additives exerted similar effects on diarrhoea incidence (Table 1) and mortality rate (data not shown) ($P>0.05$).

Conclusion

The results demonstrated that the blend of acids in OA product imposed different impact on the performance of piglets. Under practical farm conditions, synergistic blend of OA (SGG and SPH) are more efficacious than single OA (butyrate) in improving growth. The data suggest the application of SGG and SPH to support production goals of piglets raised without AGP under commercial farm conditions.

Table 1. Growth performance and incidence of diarrhea in piglets fed with basal diet (NC), antibiotic (AGP) and synergistic blend of OA from d1–35.

TREAT	ADFI, g	ADG, g	FCR, kg/kg	Diarrhea incidence, %
NC	517^{d}	327^{d}	1.59^{a}	10.7^{a}
AGP	565^{a}	399^{a}	1.42^{d}	2.7^{c}
Butyrate	547^{bc}	368^{c}	1.49^{b}	5.5^{b}
SPH	544^{c}	375^{bc}	1.45^{c}	4.4^{b}
SGG	552^{b}	379^{b}	1.46^{c}	4.3^{b}

^{a,b}Means within a column having a different letter differ significantly ($P<0.05$).

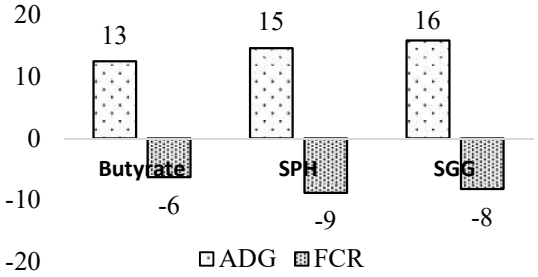

Figure 1. Percent improvement in the performance of piglets given a synergistic blend of organic acids and butyrate in relation to negative control.

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Swine producer needs for fresh on-site mixing of PCV-2 / M. hyo vaccines in USA, Germany and China

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Introduction

The fresh mixing of PCV2 and M. hyo vaccines for combined injection in swine was first introduced in 2009 by Boehringer Ingelheim to reduce stress through less injections in piglets and lower the workload for swine producers. To date, the fresh mixing of PCV2 and M. hyo vaccines requires a transfer needle for vaccine liquid transfer.

Boehringer Ingelheim is developing a new PCV-2 / M. hyo vaccine mixing system (TwistPak®), aiming to provide a safer and more efficient mixing procedure retaining the flexibility of using the products as a monovalent or combined vaccine. For developing a system that is in line with the needs and expectations of veterinarians and swine producers, information on the importance of factors in the mixing process needed to be gathered from future users.

Materials and Methods

An empirical market research study was conducted with a total of n=131 swine production owners and farm staff veterinarians across Germany (n=50), USA (n=31) and China (n=50). Interviews were conducted in person from September through December 2019 using a standardized questionnaire asking potential users of swine vaccine mixing systems how relevant a pre-specified set of mixing system characteristics is to them on a 10-point rating scale, where 1 means "Not at all relevant to me" and 10 means "Highly relevant to me". Results show mean values across respondents who indicate the respective system characteristics to be very be important (top-2 box, 9+10). Significance of comparisons between countries are based on two-tailed Z tests ($p \leq 0.05$).

Results

Vaccination handlers expect systems to provide in first place confidence that the substances are properly mixed, to allow for clean and hygienic mixing as well as safe and compliant handling (cf. Table 1).

Relevant differences between countries exist as far as user safety and ease of handling are concerned. Both are perceived to be very important in Germany but less in the US. Injection handlers in China also have a high demand for user safety and on top of that prefer having not too many system parts and handling steps, which in Germany is not as much an issue. Design has a moderate importance in China but significantly less in Germany and the US.

Table 1. % of respondent with strong agreement on relevance of mixing system attributes (9+10 on scale)

	Total (n=131)	A) US (n=31)	B) DE (n=50)	C) CN (n=50)
Confidence, substances are mixed properly	73%	71%	82%	66%
Clean preparation and hygienic mixing	69%	68%	80%	60%
Safety for user	68%	**45%^{B}**	**80%^{A}**	70%
User Compliance	63%	55%	68%	64%
Time to mix substances	56%	45%	62%	56%
Very low risk of product loss	55%	48%	68%	46%
Easy to handle	55%	**32%^{B}**	**70%^{A}**	54%
Not too many handling steps	50%	52%	**36%^{C}**	**64%^{B}**
Not too much waste	50%	42%	44%	60%
Not too many parts	47%	52%	**30%^{C}**	**62%^{B}**
Appealing / innovative design	24%	**16%^{C}**	**8%^{C}**	**44%^{AB}**

[2] Capital letters indicate significant differences at $p \leq 0.05$ level

Conclusions and Discussion

A mixing device that meets the needs across markets should primarily enable a proper, guaranteed failsafe and hygienic mixing of vaccines.

However, partly significant differences between the countries show that it would be wrong to focus just on these attributes. Production scale is assumed to be one of the reasons why the focus in US production systems (integrated) is more on technical efficiency rather than handler safety and convenience. These latter may still be important, but sufficiently well controlled with current solutions and perceived as no pain points. Respondents from China may have experienced that the delegation of pig medication works better with only few handling steps and system parts to be handled whereas in Germany delegation is happening less frequently as operations are smaller and the division of labor is less complex.

It is worth pointing out that the waste issue is not neglected in any country and the least in China.

Local training materials and brochures are currently created by Boehringer Ingelheim in line with pig producers' needs identified in this study.

Development and characterization of house fly larvae (*Musca domestica*) meal for future use as pig feed

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Introduction

The urgent need for sustainable protein sources has increased research on insect proteins, being the most studied for animal feeding the black soldier fly and tenebrio molitor (1). The house fly is an undesirable plague in animal productions, but its larvae could become an alternative to feed animals. Thus, the objective of this work was to develop and characterize house fly larvae meal (HLM) and evaluate its amino acidic contribution to weaned pigs.

Materials and Methods

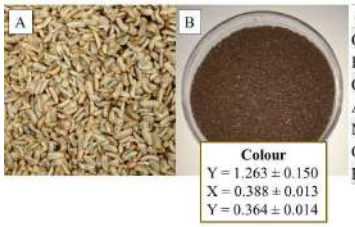
The house fly larvae were collected from a farm of laying hens in the Metropolitan Region, Santiago, Chile. The larvae were separated from the organic matter, washed, dried in an oven (100°C/3 h) and crushed to obtain the meal. The HLM was characterized by appearance, color (Konica- Minolta CR- 300, Japón), proximal chemical analysis (2), calcium and phosphorus content (2), fatty acid (3) and amino acid profile (4), amino acid score for weaned pigs according to the recommendations of the National Research Council (5), Oxygen Radical Absorbance Capacity (ORAC) (6), and microbiological analysis (7). Descriptive statistics are used for the analysis of the results. The analyses were carried out in quintuplicate.

Results

The larvae were white with brown spots (Figure 1A), and the HLM was brown (Figure 1B), possibly due to the effect of non-enzymatic browning by drying. The main components of HLM were protein and ether extract, as reported by other authors on fly meal (1). The ratio Ca:P was 1:1, considered suitable for animal feeding (Figure 1) (8). The antioxidant capacity was 8,736 ± 1,010 µmol TE/100g, which is considered high, compared to other animal feeds. The amino acid profile was characterized by high contents of aspartic-glutamic acids (8.8±0.7%), sulfur-containing amino acids (2.8±0.2%), alanine (2.7±0.1%) and lysine (2.2±0.1%) (Figure 2A). The amino acid profile was similar to meat meal (8). Based on the amino acids score analysis, none of the essential amino acids were limiting for weaned pigs, obtaining all scores > 1. Therefore, HLM is an excellent source of amino acids for pigs. The main fatty acids were oleic (7.1±0.4), palmitic (5.1±0.3) and linoleic (3.6±0.2 g/100g) (Figure 2B). Importantly, in terms of microbiological content, HLM is compatible with their use in feed (mesophilic aerobic: $1{,}7x10^3$ and coliforms <10 CFU/g; Salmonella: absent).

Conclusion

Considering the easy obtain of the HLM and its desirable nutritional content, we envisage this alternative protein source with high nutritional potential for pigs.



Components	Mean ± SD
Crude protein	54.8 ±1.8
Ether extract	21.7 ± 0.7
Crude Fiber	6.0 ± 0.4
Ash	7.6 ± 0.3
Nitrogen-free extract	10.6 ± 1.2
Calcium	1.2 ± 0.1
Phosphorus	1.1 ± 0.1

Figure 1. A: appearance of house fly larvae, B: appearance of house fly larvae meal (HLM) and color parameters. Proximal chemical analysis of HLM.

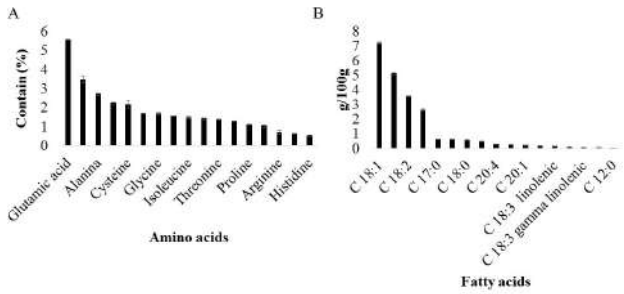


Figure 2. Total amino acid (A) and fatty acid profile (B) of HLM.

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Impact on daily growth during multiple influenza coughing outbreaks in a finishing farm monitored by SoundTalks

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Introduction

Cough is a common symptom of finishers. Nowadays, real-time technology is available for pig cough recognition as part of a precision livestock farming system[1,2]. However, once cough is recognized in the field, there is a need for a better understanding of its concurrent impact on growth performance. Therefore, the objective of this study was to compare and quantify the impact in daily growth (ADGW) of disease-coughing outbreaks occurring in a finishing batch of pigs under commercial conditions.

Materials and Methods

The study was performed in an open finishing pen provided with an optiSORT sorting gate with automatic body weight recording (Hoelscher & Leuschner GmbH & Co. KG). Cough monitors (SoundTalks NV, Belgium) were also installed, and an algorithm-based respiratory distress index (RDI) was continuously generated and aggregated daily from the farm-recorded sound. A linear mixed model was proposed to model the daily growth of influenza-free batches through a smooth function of the time and an ARMA (2,1) as the auto-correlation structure among the observations within the same curve. 95% confidence limits (95% CL) over time were estimated based on the model and used as graphical tools to evaluate whether the potential cough-affected growth curves (RDI > 1) were found within the expected bounds. Naively, the difference among curves within the influenza period was evaluated through a classical linear model to model the growth based on the time, a covariate labeling the curves' statues (baseline or target), and a covariate labeling the period's status (free or non-free of influenza). Interactions in the model were essential to evaluate the differences among these trajectories. Finally, Tukey correction was used to analyze the significance of multiple comparison.

Results

Individual ADGW data of a total of 350 pigs and the average daily RDI was recorded for 123 days. Two coughing outbreaks were detected and diagnosed as influenza by PCR, lasting 14 days and 7 days consecutively with a mean and maximum RDI of 2.50 and 4.99, and 2.50 and 3.87, respectively (Figure 1). Overall, there were no statistical difference in the curves' growth within the periods of influenza. However abnormally lower growth was detected during the first outbreak impacting severely the ADGW. This effect was not observed during the second outbreak mitigating the effect of the first outbreak as an overall.

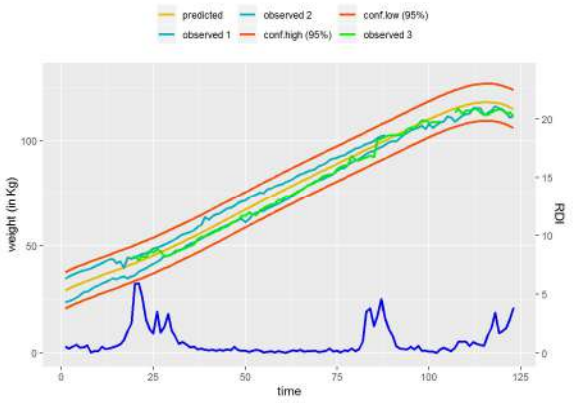

Figure 1. Non-affected (light-blue lines), cough-affected (light-green line), predicted (yellow line) and 95% CL (red lines) growth curves of the study. RDI values overtime (dark blue line).

Conclusions and Discussion

Results of this study highlight the fact that even mild clinically cough episodes could potentially impact performance parameters such as ADGW. Cough-affected curve remained within the lower CL of the reference growth curve potentially due to the small sample size of the study (wide 95%CL). There are not statistical differences in the curves' growth within the periods of influenza potentially due to the small numbers of days affected, and because the second outbreak mitigated the effect of the first one. Nevertheless, this is the first time that the impact of cough in growth performance in a commercial finishing farm is evaluated with a statistical model, however more data are needed to obtain enough power to detect even small differences among curves.

Acknowledgments

The authors would like to thank all the farm personnel involved in the study.

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Comparison of growth performance, reproductive organs and carcass characteristics between entire male pigs and male pigs immunologically castrated with two different products

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Introduction

Physical castration of young male pigs to prevent boar taint in pork meat is common industry practice, but increasingly controversial due to animal wellbeing concerns (1). Immunization against GnRF is an effective alternative to prevent boar taint (2,3), which also offers production benefits as pigs remain functional males, with their natural steroid hormones and growth efficiency, until late in life. This study looks at the comparative effect of two different anti-GnRF immunological products on multiple in vivo and post-mortem parameters.

Materials and Methods

A total of 44 pigs were randomly allocated to one of three groups: 14 entire males received placebo (EM), 14 received Improvac® (IM1) and 16 an alternative product (IM2). All pigs received two doses, first (V1) at 14.5+2.9 kg and the second (V2) 10 weeks later at 90.9+9.9 kg. Four weeks after V2 pigs were slaughtered (132.4+8.1 kg). Animals were scanned at V2 with computed tomography to determine testis volume (4). After slaughter fat thickness and lean meat content were measured with Fat-O-Meat'er II (Frontmatec, DK). Bulbourethral glands were weighed and measured, and seminal vesicles were weighed. Statistical analyses were performed with the GLM procedure of SAS software.

Results

Feed consumption was significantly higher in IM1 and IM2 than EM between V2 and slaughter. Feed conversion was higher (P<0.05) in both IM1 and IM2 from V2 to slaughter and for the total study. Testis volume at V2 was lower (P<0.05) in IM2 than IM1 and EM. At slaughter it was significantly higher in EM than IM1 with both significantly higher than IM2. Bulbourethral glands were significantly heavier in EM than IM1 and IM2 and numerically, but not significantly, longer. Seminal vesicle weight was significantly higher in EM than IM1 and IM2. Testosterone was lower (P<0.05) in both IM1 and IM2 than EM at slaughter and tended to be lower in IM2 (P=0.06) at V2. No significant differences in carcass weight were obtained but carcass yield was significantly lower in EM than IM1 and IM2, probably because of larger testis size and because flare fat weight (absolute and relative) was significantly lower in EM. No differences in fat depth and lean meat content were obtained.

Conclusions and Discussion

This preliminary work shows that both vaccines effectively castrated pigs, increased the feed consumption after second dose, reduced final testicle size, bulbourethral glands and seminal vesicles. They also increased carcass yield and did not affect carcass quality characteristics. However, both testicle size and testosterone concentration were lower in IM2 before the second dose, with the former being statistically significant and the latter nearly so, suggesting some early onset of immunological activity after the first IM2 dose.

Table 1. Carcass, production and sex gland characteristics by treatment[+].

	TREATMENT			
	EM	IM1	IM2	P-value
Live weight, kg				
V1	14.2	14.7	14.6	0.907
V2	88.3	93.1	91.2	0.443
Feed consumption, kg/d				
V1-V2	1.9	2.0	1.9	0.287
V2-slaughter	3.0^{b}	3.6^{a}	3.6^{a}	<0.001
Total	2.2^{b}	2.5^{a}	2.5^{a}	0.001
Conversion factor, kg/kg				
V1-V2	1.8	1.8	1.8	0.811
V2-slaughter	2.3^{b}	2.7^{a}	2.7^{a}	0.003
Total	1.9^{b}	2.1^{a}	2.1^{a}	0.002
Testis volume, cm^3				
V2	525.5^{a}	504.9^{a}	381.5^{b}	0.004
Slaughter	895.9^{a}	346.8^{b}	236.5^{c}	<0.001
Bulbourethral glands				
Weight, g	78.0^{a}	34.2^{b}	18.8^{c}	<0.001
Length, mm	11.7	9.9	9.7	0.525
Seminal vesicle				
Weight, g	195.9^{a}	28.5^{b}	11.6^{b}	<0.001
Testosterone, ng/ml				
V2	3.9	4.8	2.2	0.065
Slaughter	16.5^{a}	0.4^{b}	0.4^{b}	<0.001
Carcass				
Weight, kg	106.9	111.7	108.9	0.169
Yield, %	81.8^{b}	82.5^{a}	82.9^{a}	0.002
Flare fat, kg	1.4^{b}	1.9^{a}	1.9^{a}	<0.001
Flare fat, %[1]	1.0^{b}	1.5^{a}	1.5^{a}	<0.001
Fat depth[2]	22.2	23.9	24.0	0.508
Lean, %[3]	56.5	54.8	54.8	0.529

[+] Different superscript in a row indicate significant (P<0.05) differences between treatments; [1] Relative flare flat with respect to live weight; [2] Fat thickness between 3rd and 4th last ribs and at 6 cm of the midline and lean meat percentage measured with Fat-O-Meat'er II.

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Improvac® can Temporarily Suppress Estrus in Gilts Intended for Slaughter

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Introduction

Improvac® (GnRF analog-DT conjugate) is an immunological product that is used as a safe and effective alternative to physical castration of male pigs. It is used to manage unpleasant aromas ('boar taint') that can occur during cooking and eating of pork from male pigs as well as to eliminate the risk of infection or mortality associated with physical castration. Improvac® works by stimulating the immune system of the pig to produce antibodies that bind to its own gonadotropin releasing factor (GnRF), which leads to suppression of the hypothalamic-pituitary-gonadal (HPG) endocrine axis (immunological castration). Use of Improvac® allows male pigs to be raised intact for most of their growing period with the benefit of substantially improved feed/gain efficiency. The objective of this study was to determine if Improvac® can temporarily suppress estrus in gilts intended for slaughter when administered at 9 and 20 weeks of age and slaughtered at 30 weeks of age.

Materials and Methods

Forty (40) gilts sourced from a conventional farm were enrolled in the study and randomly assigned to one of two treatment groups (20 gilts per group), either Saline (CP; T01) or Improvac® (IVP; T02), administered 2 mL subcutaneous at 9 and 20 weeks of age (Days 0 and 77), using a generalized block design with blocks based on body weight. Two gilts (one per treatment group) were removed from the study for health issues prior to the second treatment administration. This study was conducted under the direction of the study site's IACUC and complied with all applicable animal welfare regulations.

From two weeks prior to the second treatment administration (Day 63) through 30 weeks of age (slaughter; Day 147), the gilts were observed daily for detection of estrus using the back pressure test in the presence of a mature boar (standing response). Detection of estrus was performed by personnel experienced and trained in the practice. The boar was led past the gilt pens and allowed to have fence line (head to head) contact; the back pressure test was then followed. The flank of each gilt was rubbed, and the back pressed. Gilts were deemed to be in estrus when they responded to the back pressure test with a frozen stance, arched back and cocked ears; e.g. the standing response. The following scoring system was used:
0 = No Estrus Observed
1 = Estrus Observed

Results

Frequency distribution of estrus detection (Days 91-147) are shown in Table 1. The percent of Improvac®-treated gilts (T02) with estrus detected at least once was significantly (P=0.0057) lower than the percent of Saline-treated gilts (T01) with estrus detected at least once beginning two weeks post second treatment.

Table 1. Frequency Distribution of Estrus Detection Days 91-147.

	No Estrus Detected		Estrus Detected At Least Once	
Treatment	N	%	N	%
Saline	8	42.1	11	57.9
Improvac®	18	94.7	1*	5.3

*8 weeks after second dose.

Conclusions and Discussion

Under the conditions of this study, the suppression of estrus in gilts was successfully demonstrated over a period of 8 weeks (from 22 weeks of age to 30 weeks of age) when Improvac® was administered at 9 and 20 weeks of age.

Acknowledgments

Dr. Scanlon Daniels, Circle H Headquarters, Dalhart, TX.

Automated magnetic bead-based extraction of 1 to 48 samples using the new IndiMag 48

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Introduction

Using magnetic-bead technology, we developed the IndiMag Pathogen Kit (previously: MagAttract 96 cador Pathogen) for use on different automated platforms. The kit can be used for extraction of viral RNA and DNA and bacterial DNA from a large range of porcine, bovine and avian samples. IndiMag 48 is a new instrument for magnetic bead-based extraction of nucleic acids. Designed to be as fast and reliable as currently available platforms, but with greater flexibility and usability, the IndiMag 48 can process between 1 and 48 samples.
In this study, we evaluated the reliability of IndiMag 48 extraction protocols for RNA and DNA from veterinary samples.

Materials and Methods

We evaluated the performance of the IndiMag Pathogen Kit, comparing the 4-step protocol of the IndiMag 48 and the 5-step protocol of the KingFisher Flex System versus results obtained with a well-known column-based method. Nucleic acids were extracted from swabs, allantois fluid, serum, blood, tissue and fecal samples.
The purified nucleic acids were analyzed using real-time bactotype and virotype PCR/RT-PCR kits from INDICAL, detecting different viral pathogens (Influenza A virus, BVDV, SBV) and bacteria (*Mycoplasma gallisepticum/synoviae, Mycobacterium avium spp. paratuberculosis*).

Results

12 swab samples spiked with *Mycoplasma gallisepticum* (ATCC25204, n=3), *Mycoplasma synoviae* (ATCC19610, n=3) and MS-H vaccine strain (Vaxsafe Ms vaccine, n=6) were tested.
Comparable results were obtained for Mg- and Ms-positive samples tested with bactotype Mycoplasma Mg/Ms PCR Kit using both the IndiMag 48 and the KingFisher Flex System protocols.
Mg and Ms positive swabs showed improved results for samples processed using the IndiMag Pathogen Kit on IndiMag 48 versus the column-based method (QIAamp DNA Mini Kit, QIAGEN).
Eight characterized Influenza A positive allantois fluid samples tested using virotype Influenza A RT-PCR Kit (INDICAL) also showed comparable results with both the IndiMag 48 and the KingFisher Flex System protocols. Slightly better results were obtained with four samples using the manual extraction method (QIAamp Viral RNA Mini Kit, QIAGEN).
Further data will be shown for RNA virus-positive serum and blood (BVDV, n=16), tissue samples (SBV, n=14) and fecal samples positive for Mycobacterium avium ssp. Paratuberculosis (n=13), which indicate equivalent or better performance with IndiMag 48 versus KingFisher instruments.

Conclusions and Discussion

Magnetic bead-based nucleic acid extraction is generally faster, easier to automate and better suited to high-throughput testing. The IndiMag Pathogen Kit demonstrated comparable performance versus a well-known, column-based extraction method. On the new IndiMag 48 extraction platform, it showed equivalent or better results compared with the Kingfisher platform.
IndiMag 48 supports cost-effective nucleic acid extraction with reduced plastic waste. It comes with pre-loaded protocols for automation of the IndiMag Pathogen Kit and offers the possibility to add more protocols via a touchscreen. IndiMag 48 is self-contained, requiring no additional software or hardware to create or edit individual protocols, and it has a small footprint, suitable for small labs. The run time and result reliability are comparable to or better than those obtained with the 96-well platform assessed here. IndiMag 48 offers a high-quality, reliable option for nucleic acid extraction with high potential for cost savings and plastic waste reduction.

The use of *Lawsonia intracellularis* qPCR on ileum tissue is a good and reliable alternative for imunohistochemistry

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Introduction

To confirm the diagnosis of Porcine Intestinal Adenomatosis (PIA) due to infections *with Lawsonia intracellularis (Li)*, the current golden standard is the combination of HE-histology in combination with detection of the presence of *Li* bacteria by immunohistochemistry staining (IHC). The objective of this study is to test whether the use of a *Li* qPCR test is an alternative for histology and subsequent IHC testing.

Materials and methods

At a routine slaughterhouse investigation, intestines were taken from pigs from eight Dutch farms. At slaughter, 40 ilea were collected. Out of the 40 ilea, in total 10 ilea were selected for histological analysis based on detection of macroscopic evidence of mucosal thickening. Samples were taken approximately 10 cm before the ileocecal junction and immediately fixated in formalin for histology (IVD laboratory). Adjacent to the tissue cut for histology, a small piece of tissue (width of approximately 3-5 mm) was frozen for subsequent Li qPCR analysis (Bioscreen, Hannover).

Results

In total 79 samples could be analyze by both qPCR and IHC. From 2 farms all obtained samples were negative for IHC as well as qPCR. Data of the comparison of qPCR and IHC is presented in figure 1. In total 25 ilea samples were negative for both IHC as for qPCR. All negative samples in figure 1 are visible as one dot. In total 27 out of the 79 samples (34%) were positive for IHC, which were all positive for PCR as well. When considering the PCR either positive or negative, a sensitivity of 100% and a specificity of 49% could be obtained (Table 1). To improve the specificity, qPCR cut off values were set at <5 log GE/ml (negative) or >6.0 log GE/ml (positive). Samples between log 5.0 and 6.0 log GE/ml are considered inconclusive (n=16; 7 pos IHC). The use of these cut off values improved specificity to 93% with a minor loss of specificity towards 95% (Table 2).

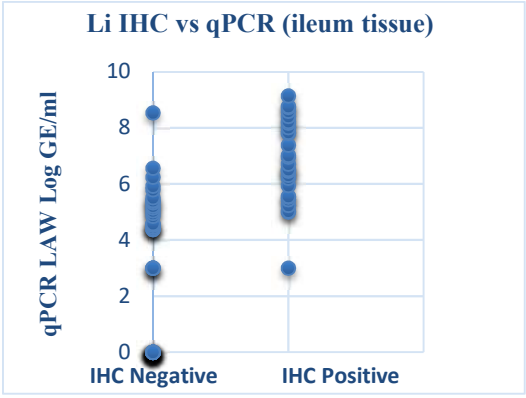

Figure 1 IHC vs qPCR results

Table 1 PCR vs qPCR resulted in a sensitivity of 100% and specificity of 49% .

		IHC	
		Pos.	Neg.
qPCR	Pos.	27	26
	Neg.	0	25

Table 2 The use of cut off values of <5 and >6 log GE/ML improved specificity to 93% with a sensitivity of 95%.

		IHC	
		Pos.	Neg.
qPCR	Pos. (>6 Log GE/ml)	19	3
	Neg. (<5 Log GE/ml)	1	39

Discussion

All of the obtained samples were taken in the slaughterhouse. While the most pragmatic hygiene protocols in these circumstances was taken to prevent cross contamination, it cannot be completely excluded. This might be the cause of one of the outliers (IHC negative but had a high *Li* GE/ml, which is visible Figure 1. However, even with these outlier a high sensitivity of 95% and specificity of 93% could be obtained.

Conclusion

The use of qPCR is a good alternative for the costly and time-consuming IHC. This data shows that ileitis due to infections with Lawsonia intracellularis can be detected at slaughter using qPCR technique.

Assessing survival chances of neonatal piglets in field conditions

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Introduction

The causes of decreased birth weight (BW) and their impact on piglet survivability are extensively documented (1), yet using only birth weight as an indicator for survivability may be too simplistic (2). Piglets born immature at term are unlikely to survive (3). However, birth weight is a very easy, fast and non-invasive method of assessment. A threshold of 1.13 kg was proposed below which a piglet has lower survival chances until weaning (4). Head shape and vitality scores were used to define Intra Uterine Growth Restriction (IUGR) in piglets (3). These scoring systems are subjective and little is known about the genetic line component.

The objective of this study was to compare different survival criteria in different genetic lines in order to provide a universal advice for survival chance assessment in field conditions.

Materials and Methods

Data were collected from three farms located in Belgium (A and B) and Spain (C) with two different genetic lines (Danbred (2 farms) and Topigs (1 farm) represented. All live born piglets (BA) from 80 litters were assessed. The piglets were processed between 2 and 24 hours after birth. BW, rectal temperature (RT) and Crown Rump Length (CRL) were measured. A morphological IUGR score was based on 3 criteria: dolphin head shape, bulging eyes and wrinkles perpendicular to mouth (2). When at least one of the three criteria was met, the piglet was considered to be IUGR. Body Mass Index (=BW/(CRL)²) was calculated. From farm C, piglet pre-weaning mortality data were collected. Statistical analysis was performed using mixed or glimmix procedures where appropriate and Pearson correlation coefficients were calculated between the different parameters.

Results

There was a clear difference between genetic lines for litter size, BW, BMI and IUGR incidence (Table 1). The incidence of IUGR was higher when the average birth weight was lower but the incidence of IUGR in the low weight piglets (<1.13kg) was remarkably similar between farms. Piglet mortality was high in low weight piglets specifically when they also suffered from IUGR (Table 2). There was a farm and weight effect for RT, but within the C farm, piglets that died had lower RT compared to those surviving.

The highest correlation with BW was CRL (R = 0.89, 0.87 and 0.70 for farm A, B and C respectively). IUGR was negatively correlated to BW (R = -0.51, -0.68 and -0.43 for farm A, B and C respectively).

Table 1. Litter data and IUGR data per farm

	Farm A	Farm B	Farm C
Litters (n)	15	15	50
Genetic line	Danbred	Danbred	Topigs
Parity	2.33	5.07	3.78
BA per litter	18.73[a]	17.20[a]	14.10[b]
litter weight BA (kg)	21.25[a]	20.17[a]	20.85[a]
Average per litter			
BW (kg)	1.20[a]	1.18[a]	1.51[b]
RT (°C)	38.18[a]	37.71[a]	37.92[a]
CRL (cm)	26.6[a]	25.05[b]	24.51[b]
BMI	16.69[a]	18.37[a]	25.08[b]
IUGR incidence per Farm			
All piglets	41.3[a]	42.6[a]	25.0[b]
BW < 1,13 kg	76.6[a]	76.4[a]	69.9[a]
BW ≥ 1,13 kg	21.4[a]	11.7[b]	17.4[ab]

[a] and [b] indicate statistical differences ($p \leq 0.05$) between farms.

Table 2. Farm C individual piglet data.

Weight threshold (kg)	IUGR survived		IUGR died	
	< 1.13	≥ 1.13	< 1.13	≥ 1.13
Piglets (n)	45	93	27	11
BW (kg)	0.93[a]	1.48[1]	0.81[b]	1.52[1]
RT (°C)	37.92[a]	38.10[1]	34.55[b]	36.07[2]
CRL (cm)	20.95[a]	24.41[1]	20.65[a]	24.78[1]
BMI	21.34[a]	24.89[1]	19.28[b]	24.91[1]
	Normal survived		Normal died	
Piglets (n)	26	460	5	33
BW (kg)	1,06[c]	1,62[3]	0,91[d]	1,46[4]
RT (°C)	37.65[c]	38.31[3]	32.00[d]	35.31[4]
CRL (cm)	22.10[c]	24.97[3]	23.80[d]	25.53[3]
BMI	21.81[c]	26.11[3]	16.01[d]	22.60[4]

For < 1.13 kg pigs, [a] and [b] or [c] and [d] indicate statistical differences ($p \leq 0.05$) ; For ≥ 1.13 kg pigs, [1] and [2] or [3] and [4] indicate statistical differences ($p \leq 0.05$)

Discussion and Conclusion

BW remains a unversal criterion to assess survival chances in field conditions. Low BW piglets demonstrating IUGR characteristics and piglets with a low RT (< 36°C) between 2 and 24 hours after birth are in a critical situation. They should receive special care or be euthanized for welfare reasons.

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Using technology to assess labor efficiency in US swine farms

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Introduction

The swine industry has gone through significant changes over the last several years, with a movement towards larger and specialized farming systems[1]. Such shift in production has led producers to hire personnel as they can no longer rely solely on family members as their main labor source. Even though we had advancements in record-keeping, benchmarking, swine nutrient requirements, biosecurity, and performance-enhancing technologies[2], our understanding of labor efficiency in swine farms is lacking. The objective of this prospective cohort study was to use beacon-sensing technology under field conditions to i) describe movement patterns of workers inside swine farms, and ii) to investigate whether time spent moving between rooms and time spent in certain farm areas by role (managerial positions versus other staff) are associated with number of pigs weaned per sow.

Materials and Methods

Three farrow-to-wean farms located in the United States Midwest were enrolled in the study from March 2018 to April 2019. Each farm had an internal biosecurity system (B-eSecure, PigCHAMP Pro Europa) capable of tracking the presence of Bluetooth beacons on each of the farm's rooms. A unique Bluetooth beacon was assigned to each person working on the farm. Data was collected and sent to a central database through Wi-Fi in real-time for a period of one year. Movement and production data were aggregated at the week level and analyzed using Stata-IC 14. To understand the impact of movement patterns on the number of pigs weaned per sow, both descriptive statistics and linear regression models for each farm were used. Predictors of interest included time spent moving between rooms for managers and other staff, time spent in rooms for managers and other staff, the number of pigs weaned per sow in the previous week, pre-weaning mortality, and season. Statistical significance was declared at $P < 0.05$, a tendency was declared at $0.05 \leq P < 0.10$.

Results and Discussion

A total of 24, 21 and 47 unique types of movement were detected on farms 1, 2 and 3, respectively. The median time to move between two different rooms was numerically higher for farm 2 compared to farms 1 and 3 (Table 1). Median time spent in rooms was higher for the managerial position worker on farm 2 (median 19.5 minutes spent in destination room for managers versus 10 minutes for other staff), but similar for farms 1 (median 7 minutes for both manager and staff positions) and 3 (median 7 minutes for staff and 6 minutes for manager). Results from final statistical models showed that for farm 1, a five-hour increase in time spent in rooms by the manager was associated with an increase in one weaned piglet for every 10 litters ($P = 0.01$). This finding is not surprising considering that managers are normally working in daily chores and not only in administrative tasks for smaller farms such as farm 1. For farm 2, an increase in time spent working in the farrowing rooms of approximately two hours per worker per week tended to increase the number of weaned piglets by one piglet for every 4 litters ($P = 0.087$). This finding could be due to an increase in pig care, which would likely lead to a higher number of weaned animals.

Table 1. Basic descriptors for farms enrolled in the study

	Farm		
Descriptor	1	2	3
Size (number of sows)	1,400	4,400	4,500
Number rooms	18	25	26
Number workers	15	24	35
Time spent moving between rooms[1] (minutes)	1 (1)	5 (18)	1 (3)
Time spent in rooms (all) in minutes[1]	7 (11)	11 (30)	7 (13)

[1]Values expressed as median (inter-quartile range) due to the skewed nature of the data

Conclusion

Labor efficiency is an important part of swine production both from the economical and animal welfare perspectives. This project showed that the use of technology could be an important asset in assessing labor trends and efficiency on-farm.

Acknowledgments

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Growth performance of fattening gilts, immunologically castrated using Vivax® and fed either ad libitum or on a restricted feeding regimen before slaughter at 24 weeks of age

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Introduction

As in males, immunocastration (IC) with Vivax® in females is associated with an increase in appetite and weight gain, offering potential production benefits. The profitability of these, however, depends on whether feed efficiency can also be maintained or even improved. When consumption is high feed restriction is a common management practice to optimize financial results. This abstract describes one of a series of studies to investigate the impact of IC timing and feeding regimen on gilt growth performance.

Materials and Methods

480 gilts were randomly allocated to 8 groups of 60 at 12 weeks of age. 4 groups (T1, T3, T5, T7) were fed *ad libitum* and 4 (T2, T4, T6, T8) were feed restricted. Groups T3 to T8 all received two doses of Vivax® with the second (V2), which produces IC, at 4 (T3, T4), 6 (T5, T6), or 8 (T7, T8) weeks before slaughter at 24 weeks of age. T1 and T2 groups remained untreated and no placebo injections were given. Gilts were weighed weekly and feed consumption per pen was recorded daily, with average feed intake per pig calculated weekly. For groups T2, T4, T6 and T8 feed was restricted to 2.8kg/head/day starting either at the time of V2 or, for the untreated control group T2, at the same time as T8.

Results of Average Daily Feed Intake (ADFI) and body weights were analyzed using a general linear mixed model with repeated measures. Average Daily Gain (ADG derived from body weights analysis), ADFI and Feed Conversion Ratio (FCR=Average Daily Feed Intake / ADG) were analyzed for the period between V2 and slaughter and the overall study period (12-24 week).

Results

Table 1 shows the results for ADFI, ADG, and FCR for the period between V2 and slaughter. In each case pair-wise statistical comparisons are shown between the IC group and the negative control receiving the same feeding regimen (*), and between treated groups receiving the same immunization protocol but different feeding regimens (+).

Table 2 shows the same parameters results for all treated groups for the entire fattening period (12 to 24 weeks of age), with the addition of final bodyweight. There were no statistically significant differences in performance prior to V2 so the overall results essentially represent the dilution of the post-V2 effect over a longer period.

Table 1. V2 to slaughter growth performance of IC gilts with either ad libitum or restricted feeding.

	ADFI (kg)	ADG (kg)	FCR
V2-slaughter period 4 weeks			
T1 (ad lib)	2.94++	0.966	3.08++
T2 (rest.)	2.65++	0.979	2.72++
T3 (ad lib)	3.29**++	1.148**++	2.87+
T4 (rest.)	2.71++	1.060**++	2.57+
V2-slaughter period 6 weeks			
T1 (ad lib)	2.77++	0.969	2.88++
T2 (rest.)	2.55++	0.985	2.61++
T5 (ad lib)	3.19**++	1.079**	2.95++
T6 (rest.)	2.70*++	1.054**	2.56++
V2-slaughter period 8 weeks			
T1 (ad lib)	2.59+	0.961	2.71+
T2 (rest.)	2.43+	0.965	2.52+
T7 (ad lib)	2.93**++	1.046**	2.81*+
T8 (rest.)	2.62**++	1.019**	2.59+

Table 2. Overall (12-24 week) growth performance of IC gilts with either ad libitum or restricted feeding.

	ADFI	ADG	FCR	Final B. Wt.
Ad lib feeding				
T1	2.32	0.939	2.47++	111.2
T3	2.40*++	0.984**	2.44++	114.9**
T5	2.52**++	0.991**	2.54*++	115.5**
T7	2.55**++	1.000**	2.55*++	116.2**
Restricted feeding				
T2	2.21	0.941	2.35++	110.4
T4	2.22++	0.961	2.31++	111.9
T6	2.27++	0.968	2.34++	112.5*
T8	2.33**++	0.975*	2.40++	113.2**

*, ** P≤0.05, P≤0.01 vs same feed, non-IC control
+, ++ P≤0.05, P≤0.01 vs equivalent IC, different feed

Conclusions and Discussion

IC consistently increased ADFI and ADG, leading to higher slaughter weights. Feed restriction consitently improved FCR, including in untreated controls. Within feeding regimen, the impact of IC on FCR was generally not significant except for the longer ad lib IC periods, where it became higher. The numerical pattern, however, fits field observations that short periods of IC can improve FCR, while longer periods will maximize weight gain but possibly result in some FCR deterioration unless animals are limit fed.

Growth performance of fattening gilts, immunologically castrated using Vivax® and fed either ad libitum or on a restricted feeding regimen before slaughter at 26 weeks of age

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Introduction

As in males, immunocastration (IC) using Vivax® in females is associated with an increase in appetite and weight gain, offering potential production benefits. Profitability, however, depends on whether feed efficiency can also be maintained or even improved. When consumption is high, feed restriction is a common practice to optimize financial results. This abstract describes one of a series of studies to investigate the impact of IC timing and feeding regimen on gilt growth performance.

Materials and Methods

480 gilts were randomly allocated to 8 groups of 60 at 12 weeks of age. 4 groups (T1, T3, T5, T7) were fed *ad libitum* and 4 (T2, T4, T6, T8) were feed restricted. T3 to T8 all received two doses of Vivax® with the second (V2), which produces IC, at 4 (T3, T4), 6 (T5, T6), or 8 (T7, T8) weeks before slaughter at 26 weeks of age. T1 and T2 groups remained untreated and no placebo was given. Pigs were weighed weekly and pen feed consumption recorded daily, with average feed intake per pig calculated weekly. For groups T2, T4, T6 and T8 feed was restricted to 2.8kg/head/day starting either at the time of V2 or, for the untreated control group T2, at the same time as T8.

Results of Average Daily Feed Intake (ADFI) and body weights were analyzed using a general linear mixed model with repeated measures. Average Daily Gain (ADG) derived from body weights analysis, ADFI and Feed Conversion Ratio (FCR=Average Daily Feed Intake / ADG) were analyzed for the period between V2 and slaughter and the overall study period (12-26 week).

Results

Table 1 shows the results for ADFI, ADG, and FCR for the period between V2 and slaughter. Pair-wise statistical comparisons are shown between the IC group and the negative control receiving the same feeding regimen (*), and between treated groups receiving the same immunization protocol but different feeding regimens (+).

Table 2 shows performance parameters for all groups for the entire fattening period (12 to 26 weeks of age).

Conclusions and Discussion

IC consistently increased ADFI and ADG, leading to higher slaughter weights. Feed restriction consitently improved FCR, including in untreated controls.

Within feeding regimen, the impact of IC on FCR was generally not significant except for the longer ad lib IC periods, where it became higher. The T4 to T2 comparison in overall FCR is potentially misleading owing to the different timings of feed restriction. Prior to V2, T4 gilts were able to eat more than T2 and did so. The general numerical pattern fits field observations that short periods of IC can improve FCR, while longer periods will maximize weight gain but possibly result in some FCR deterioration unless animals are limit fed. Maximizing the profitability of IC depends on optimizing both immunization timing and feeding regimen to suit production objectives.

Table1. V2 to slaughter growth performance of IC gilts with either ad libitum or restricted feeding.

	ADFI (kg)	ADG (kg)	FCR
V2-slaughter period 4 weeks			
T1 (ad lib)	2.83++	0.841+	3.37++
T2 (rest.)	2.57++	0.915+	2.83++
T3 (ad lib)	3.23**++	1.025**++	3.16**++
T4 (rest.)	2.61++	0.929++	2.82++
V2-slaughter period 6 weeks			
T1 (ad lib)	2.82++	0.912	3.10++
T2 (rest.)	2.56++	0.951	2.69++
T5 (ad lib)	3.30**++	1.031**	3.20++
T6 (rest.)	2.66++	1.018**	2.62++
V2-slaughter period 8 weeks			
T1 (ad lib)	2.74++	0.920+	2.99++
T2 (rest.)	2.51++	0.967+	2.60++
T7 (ad lib)	3.04**++	0.996**	3.05++
T8 (rest.)	2.57++	1.009*	2.56++

Table 2. Overall (12-26 week) growth performance of IC gilts with either ad libitum or restricted feeding.

	ADFI	ADG	FCR	Final Body wt.
Ad lib feeding				
T1	2.46++	0.946	2.61++	128.28
T3	2.54	0.990**	2.56+	132.45**
T5	2.71*++	1,000**	2.71**++	133.55**
T7	2.65*++	0.989**	2.68*++	132.43**
Restricted feeding				
T2	2.30++	0.966	2.39++	128.55
T4	2.43**	0.980	2.47**+	129.84
T6	2.39*++	0.991	2.41++	130.88*
T8	2.35++	0.990	2.38++	130.83*

*, ** P≤0.05, P≤0.01 vs same feed, non-IC control; +, ++ P≤0.05, P≤0.01 vs equivalent IC, different feed.

Practical application of quality control tool SPC-Capability analysis for evaluation of vaccination protocols in pigs

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Introduction

Statistical Quality Control Improvement philosophy refers to the use of statistical methods in the monitoring and maintaining of the quality of processes. Quality is inversely proportional to variability, if variability in a process decreases, the quality of that process increases, and the result of the decisions made based on that process will improve. Quality control improvement involves the set of activities used to ensure that the processes meet requirements and are improved in a continuous basis having Statistical Process Control (SPC) as the major tool[1]. SPC is a framework of ideas and a collection of problem-solving tools useful in achieving process stability through the reduction of variability[2]. Process capability analysis is part of this quality tool-box, and it measures how well a process performs comparing the distribution of sample values to the specification limits and target[1]. The objective of this field evaluation was to apply SPC-Capability analysis to evaluate the impact of vaccine interventions on mortality in nursery pigs.

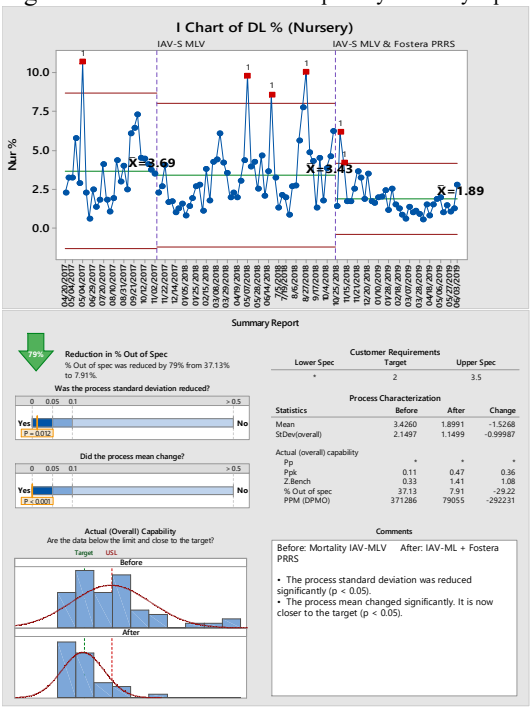
Materials and methods

This evaluation was implemented in a 1,200 sow-farm located in south-east Iowa, USA. The breeding herd was under a quarterly mass vaccination with Fostera® PRRS since 2014 achieving PRRS stability since then. Farm staff decided to implement the vaccination against Influenza-A virus with Modified Live Virus vaccine (IAV-S MLV vaccine, Ingelvac Provenza™, Boehringer Ingeheim Vetmedica, Saint Joseph MO.) at processing time (3-5 days of age) intranasally (1ml). This intervention was implemented for ~11 months and death loses % in nursery were collected for individual pig groups before and after IAV-S MLV vaccine intervention and evaluated using a Shewhart statistical process control chart. After initial analysis, it was determined that PRRS virus was circulating in the nursery and an addition of PRRS Modified Live Virus vaccine (Fostera® PRRS, Parsippany NJ) was implemented in pigs at processing time (2 ml intramuscular) and this intervention was included in the SPC control chart. After ~8 months implementing PRRS intervention, a comparison analysis from different periods and interventions (No intervention, IAV-S MLV and IAV-S MLV + Fostera PRRS) was placed using SPC control chart and kruskal wallis pairwise comparison test. In addition, a before and after process capability analysis was performed taking IAV-S MLV and Fostera PRRS as before and after periods respectively. The before and after data were transformed and passed the normality test (Anderson-Darling test), and for capability calculations, death loss target was assigned as 2% and 3.5% as upper specification. A before and after comparison capability analysis summary report was generated using Minitab software (18.1 State College PA).

Results and discussion

The comparison analysis between the three-death loss % periods showed no differences between no-intervention and IAV-S MLV (P-value=0.523) but a statistically significant reduction in death loss was observed when Fostera PRRS was implemented at processing (P-value=0.0001).

Figure 1. SPC Control chart and capability summary report.



Capability analysis comparison between IAV-S MLV and IAV-S + Fostera PRRS periods showed a significant reduction of the mean (P-value 0.012) and standard deviation (P-value <0.001) in death loss when Fostera PRRS was added, reducing by 79% the weekly groups with death loss >3.5%.

Conclusion

A statistically significant improvement (P-value= <0.015) was observed when Fostera PRRS at processing was added. Quality tools like Capability analysis are helpful to practitioners for objectively evaluate health interventions and make better decisions.

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Immunological castration of fattening gilts using Vivax® consistently results in increased feed intake and weight gain

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Introduction

Immunization against GnRF using Vivax® (Zoetis), also called Improvac®, Improvest® and Innosure®, is recently used in fattening gilts to suppress ovarian function and occurrence of estrus. As in males, immunocastration in females is associated with an increase in appetite and weight gain, offering potential production benefits.[1,2] A program of production studies provided the opportunity to look in detail at the consistency and magnitude of these effects.

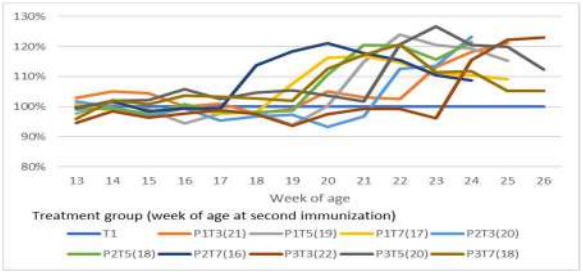
Materials and Methods

The program was conducted in three phases, each being a separate batch of gilts, with slaughter ages of 25, 24 and 26 weeks respectively. Each phase included 480 animals randomly allocated to 8 groups of 60 at 12 weeks of age. 4 (those shown) were fed *ad libitum* and 4 were feed restricted. T3, 5 and 7 all received two doses of Vivax® with the second (V2), which produces immunocastration, at 4, 6, and 8 weeks pre-slaughter respectively. Age at time of V2 consequently ranged from 16 to 22 weeks. T1 groups remained untreated. Gilts were housed by treatment in pens of 5, with pens randomly distributed. Feed consumption per pen was recorded daily and average feed intake per pig calculated weekly. Pigs were weighed weekly.

The 3 phases used different slaughter ages and were done at different times under different environmental conditions. Results of daily mean feed intake and body weights were therefore analyzed using a general linear mixed model with repeated measures by phase, respectively. The average daily weight gains were calculated from the analysis of body weights in the period from V2 to slaughter and comparison was made between treated groups and their own phase control in each phase.

Results

Figure 1 shows feed intake progression over time. Figure 1. Mean feed intake (weekly) of gilts in groups T3, T5 and T7 from phases (P) 1, 2 and 3, expressed as a percentage of the within-phase T1



All groups showed a statistically significant increase in feed intake from the second week after V2.

Table1. Summarizes the impact on weight gain. Average daily weight gain (ADG, kg) of ad lib fed gilts in the period from V2 to slaughter.

	ADG	ADG T1	% change
T3 (period 4 weeks pre-slaughter)			
Phase 1	0.958	0.883	+8.5*
Phase 2	1.148	0.966	+18.9**
Phase 3	1.025	0.841	+22.0**
T5 (period 6 weeks pre-slaughter)			
Phase 1	0.940	0.872	+7.8**
Phase 2	1.079	0.969	+11.4**
Phase 3	1.031	0.912	+13.1**
T7 (period 8 weeks pre-slaughter)			
Phase 1	0.921	0.895	+2.8
Phase 2	1.046	0.961	+8.8**
Phase 3	0.996	0.920	+8.3**

*P≤0.05, **P≤0.01.

Conclusions and Discussion

In all immunized groups feed intake rose rapidly from the second week after V2, typically peaking at over 120% of the control group after 3 to 4 weeks, but remaining substantially higher at 8 weeks. There was no obvious impact of age at V2 on the response pattern. Immunization also consistently increased ADG after V2, although less so in Phase1, where growth performance generally may have been impacted by hot and variable weather. The relative improvement appears to decrease with increasing time from V2 to slaughter, although the lower benefit is applicable to a longer period.

From a production perspective, immunocastration of gilts offers a reliable way to increase feed intake and growth. Impact on feed conversion will be discussed elsewhere and may be positive, neutral or negative depending on the balance of ADFI and ADG effects. The higer impact of IC on ADG seen with a shorter V2 to slaughter period (T3) may increase the likelihood of FCR improvement. Timing of treatment relative to slaughter and feeding regimen are both variables that can be manipulated to achieve production objectives and maximize profitability.

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Carcass characteristics of fattening gilts, immunologically castrated using Vivax® and fed either ad libitum or on a restricted feeding regimen before slaughter

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Introduction

Immunization against GnRF using Vivax® (Zoetis), also called Improvac®, Improvest® and Innosure®, is recently used in fattening gilts to suppress ovarian function and occurrence of estrus. Immunocastration (IC) in females is known to be associated with increased appetite and weight gain. After slaughter carcasses are typically heavier with more fat and lower lean meat percentage than from untreated gilts.[1] This abstract summarizes the results from a program of production studies designed to explore the magnitude of these effects in gilts with different durations of IC and fed either ad libitum or on a restricted feed regimen.

Materials and Methods

The program was conducted in three phases, each being a separate batch of gilts, with slaughter ages of 25 (P1), 24 (P2) and 26 (P3) weeks, respectively. Each phase included 480 animals randomly allocated to 8 groups of 60 at 12 weeks of age. 4 groups (T1, T3, T5, T7) were fed *ad libitum* and 4 (T2, T4, T6, T8) were feed restricted. Groups T3 to T8 all received two doses of Vivax® with the second (V2), which produces IC, at 4 (T3, T4), 6 (T5, T6), or 8 (T7, T8) weeks before slaughter. T1 and T2 groups remained untreated and no placebo injections were given. Gilts were housed by treatment in pens of 5, with pens randomly distributed. For groups T2, T4, T6 and T8 feed was restricted to 2.8kg/head/day starting either at the time of V2 or, for the untreated control group T2, at the same time as T8. Slaughter was performed according to typical Brazilian commercial practice. Carcasses were weighed head on and backfat and loin eye measured between vertebrae T14 and L1 using a Hennessey Grading Probe 7. Lean meat % was calculated using a standard formula.[2] Results of each measurement were analyzed using a general linear mixed model for each phase. Pairwise statistical comparisons were done within phase between groups on the same feeding regimen (looking at the impact of IC timing) and between equivalent IC groups on the different feeding programs (looking at the impact of feed restriction).

Results

Tables 1, 2 and 3 show results for carcass weight, backfat depth and lean meat % respectively. Different letters or the + symbol indicate significant differences ($P \leq 0.05$) across rows or between equivalent IC groups, respectively.

Table1. Carcass weights (kg) of IC and control gilts on either ad libitum or restricted feeding.

Ad lib	T1	T3	T5	T7
P1 (25w)	85.1a	86.6a	86.4a	86.1a
P2 (24w)	79.4a	81.3ab	82.5bc	83.6c
P3 (26w)	91.5a	93.6ab	95.1b	94.8b
Restricted	T2	T4	T6	T8
P1 (25w)	84.6a	84.4a	85.5a	85.2a
P2 (24w)	79.3a	79.4a	79.9a	80.9a
P3 (26w)	91.3a	91.6a	93.2a	93.6a

Table2. Carcass backfat depth (mm) of IC and control gilts on either ad libitum or restricted feeding.

Ad lib.	T1	T3	T5	T7
P1 (25w)	13.4a	15.1b	16.1b	15.8b
P2 (24w)	13.9a	14.5a	14.5a	16.0+b
P3 (26w)	15.9a	15.8a	17.1ab	18.3b
Restricted	T2	T4	T6	T8
P1 (25w)	13.7a	13.8a	14.8ab	15.7b
P2 (24w)	13.4a	13.8ab	14.3b	14.5+b
P3 (26w)	15.3a	15.9ab	16.6ab	17.5b

Table3. Lean meat (%) of carcasses from IC and control gilts on either ad libitum or restricted feeding.

Ad lib.	T1	T3	T5	T7
P1 (25w)	58.6ab	58.8b	57.8ab	57.7a
P2 (24w)	58.1a	57.6a	58.0a	57.3a
P3 (26w)	58.6c	59.2c	58.2ab	57.4a
Restricted	T2	T4	T6	T8
P1 (25w)	58.7a	58.7a	58.7a	57.8a
P2 (24w)	58.3a	57.6a	57.6a	57.6a
P3 (26w)	59.2a	58.6a	58.7a	58.4a

Conclusions and Discussion

Consistent with other reports[1], IC increased carcass weight and backfat thickness with some reduction in lean meat %. These effects typically increased with a longer V2 to slaughter interval. Except between groups T7 and T8 in P2, there were no significant differences from restricted feeding but, not surprisingly, the numerical results typically show lower values for carcass weight and backfat and a reduced IC impact. Opportunities to improve carcass value also need to be taken into account when optimizing the profitability of IC in gilts.

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Effect of attapulgite and benzoic acid addition to the diet of fattening pigs on the environmental performance of the pig live-weight at the farm gate

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Introduction

The aim of this work was to study the potential environmental impact of the dietary supplementation of attapulgite clay and benzoic acid in the diets of fattening pigs. For this purpose, an environmental Life Cycle Assessment (LCA) methodology was considered. This LCA methodology utilized the fattening pig performance results of an *in situ* experimental procedure for examining two feeding practices: the conventional diet (CNVD treatment) used by the farm and the supplementation of this diet with attapulgite clay at 4 g/kg and benzoic acid at 5 g/kg (ATTBAD treatment).

Materials and Methods

The functional unit (FU) was defined as '1 kg of pig live-weight at the farm gate' and refers to the sum of the live-weight of the finished pigs and spent sows (1). A 'cradle-to-farm-gate' analysis was performed in a commercial fattening pig farm in Greece. Emission of CH_4 from enteric fermentation was estimated by using the default Tier 1 Intergovernmental Panel of Clime Change (IPCC) emission factor (EF) (2). The default IPCC Tier 2 approach was applied for the emissions of CH_4 and direct N_2O from on-farm slurry management (OFSM). Indirect N_2O emissions were estimated with the default Tier 1 IPCC EF (2). The default European Monitoring and Evaluation Programme Guidebook (EMEP/EEA) Tier 2 approach (3) was utilized for the evaluation of NH_3. Modeling of the material and electricity inputs' supply chains was based on secondary Life Cycle Inventory datasets (Ecoinvent v. 3.4, Agri-footprint v. 4.0 and Agribalyse v. 1.3 databases as available in the SimaPro v. 8.5.2. PhD software).

Results and Discussion

Table 1 presents the potential estimates per FU for the environmental impact category indicators (EICIs) assessed. The ATTBAD system seems to be associated with an improved environmental performance as compared to the CNVD system.

The pig farm was the most important contributor to GWP-100, AE-A and AE-TE. Furthermore, maize grain supply was the most relevant for the FNMC,
EP and ARDF while the barley grain supply for the FPFC, LOP and UDP. Finally, soybean meal supply was the most important contributor to the GWP-100 (dLUC).

Table 1. Potential EICI estimates per functional unit

Environmental Impact Category Indicators	System	
	CNVD	ATTBAD
GWP-100 (g CO_2-eq)	4147.16	3847.21
GWP-100 (dLUC) (g CO_2-eq)	1179.32	1091.22
AE-A ($mmol_c$ H^+ eq)	99.67	90.77
AE-TE ($mmol_c$ N eq)	419.74	381.74
FPFC (g P eq)	1.56	1.44
FNMC (g N eq)	34.14	31.35
LOP (m^2×yr cropland eq)	6.57	6.04
UDP (m^3 world eq)	86.51	79.31
ARDF (MJ)	25.31	23.85

GWP-100: Global Warming Potential (100 years' time frame); GWP-100 (dLUC): Global Warming Potential due to dLUC; AE-A: Accumulated Exceedance – Acidification; AE-TE: Accumulated Exceedance – Terrestrial Eutrophication; FPFC: Fraction of P reaching freshwater end compartment (Freshwater Eutrophication); FNMC: Fraction of N reaching marine end compartment (Marine Eutrophication); LOP: Agricultural Land Occupation Potential (Land Use); UDP: User Deprivation Potential (Water Use); ARDF: Abiotic resource depletion-fossil fuels

Conclusions

The improved feed efficiency and total weight gain in the fattening stage associated with the supplemented diet ATTBAD were decisive factors for the potentially improved environmental performance per functional unit produced.

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Improvement in herd health and productivity in herds with swine respiratory disease with tilmicosin aqueous concentrate in lactating sows

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Introduction

Tilmicosin has been shown to effectively control respiratory disease in sows. The effect of administering Pulmotil® (tilmicosin) aqueous concentrate (PAC) via drinking water to sows during lactation is unreported. The study objective is to evaluate the health impact of the PAC lactation medication program on sow and piglet health and performance as an alternative to feed-based delivery**.**

Materials and Methods

Four 2,600 sow farrow-to-wean farms reporting sow and piglet respiratory disease caused by *Mycoplasma hyopneumoniae* and *Pasteurella multocida* and exacerbated by Porcine Reproductive and Respiratory Syndrome virus infection were enrolled. Three treatment and control sow groups were included per farm totaling approximately 1,200 sows per treatment. Treatment groups received PAC via drinking water at a rate of 2 grams/sow/day beginning 3 days prior to expected farrowing date which continued for the entire lactation period (average 20.7 ± 1.7 days). Control groups received fresh water only. Treatment integrity was maintained while implementing a McREBEL program[1,2,3,4].

Three eight rooms nursery that filled over a 2-week period received piglets from each sow farm which maintained integrity by week and by room. Twenty-four rooms, 12 with pigs from PAC treated sows and 12 from control sows, from 3 nursery sites were used during the study. Nursery facilities allowed for physical separation between treatment and control groups, however, labor was shared between treatment and control groups.

Results

Although in different stages of recovery, all sow farms were exhibiting Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) signs. The variable percent combined stillborn and mummies within the data substantiates PRRSV activity as PRRSV affects percent combined stillborn and mummy and percent pre-wean death loss, among other clinical signs[5,6,7]. Percent combined stillborn and mummies ranged from 5.76% to 13.15% for treatments and 7.46% to 12.17% for controls. Least-square mean estimates of pre-wean death loss were 13.1% for treatment vs 14.6% for controls (P<0.01). An interaction between treatment and percent combined stillborn and mummies (P=0.001) and significant treatment effects (P=0.004) were observed. Overall pre-wean death loss advantage was 1.3% for PAC treated sows. At 10% combined stillborn and mummies, the treatment vs control advantage increased to 1.95%. At 12% combined stillborn and mummies, the treatment advantage was 4.88%. The nursery death loss for pigs originating from sows treated with PAC was 5.6% vs 7.05% for control pigs (P<0.01).The odds ratio indicates control pigs are 1.28 times more likely to die (95% CI of 1.146-1.429) as compared to pigs originating from PAC sows.

Conclusions and Discussion

The study demonstrated that PAC during lactation lowered death loss. When percent combined stillborn and mummies increases, application of PAC lowered pre-wean death loss. During periods of high combined stillborn and mummies (≥ 9.5%) application of PAC can lower pre-wean death loss. Further studies will substantiate these findings and define the impact on weaning weight.

Pigs weaned from PAC treated sows had improved death loss in the nursery thus improving the odds of survival as compared to control pigs under variable PRRSV exposure.

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Identification and elimination of Swine Dysentery from a herd in a large production system

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Introduction

Swine Dysentery(SD) is classically caused by *Brachyspira hyodysentery* or other emerging brachyspira such as *Brachyspira hampsonii*. In the severe form it causes frank blood and mucus in diarrhea pigs10 weeks or older[1]. Reports of SD in the United States and Canada have increased in the last decade.[1,2,3] The long term costs and biosecurity threat of swine dysentery to large production systems makes eradication a viable option.[2,3,4] Long term control of SD has led to the evolution of multidrug resistant brachyspria.[1] Elimination programs using medication, snatiation with white wash, strict biosecurity, and rodent control are highly successful.[1,4,5] This case report details the identification and elimination of SD in a herd within a large production system.

Materials and Methods

The first indication of SD were production staff reports of higher than normal loose stools in a multisource pig flow. These quickly escalated to mucohemorrhagic stools at this site. Fecal samples were positive by PCR for *nox* and *tlyA* hemolysin genes were in this finishing sites[1]. As no clinical signs were noted in the 8 sow farms supplying pigs to the multisourced pig flow, a focused fecal collection was scheduled. Eight sow farms were tested focusing on sows with loose stool and young parity sows. 30 samples were collected and pooled by 5 for anaerobic culture and PCR.

One 35000 head four barn site in a low pig dense are was diagnosed as SD positive via fecal PCR as described above. One 2500 head breed to wean sow farm was identified as infected via testing.

Because of the potential spread and estimated economic impact of $10 to $12 (USD)[2,3,4] per finishing pig, the following interventions were considered: pig flow segregation, sow herd depopulation and medical elimination were evaluated. Segregation would significantly slow the wean to finish barn and site fill time because of the large numbers of pigs required. Depopulation cost and pig flow impacts were negative considerations. Although medical elimination requires extensive cleaning coordinated during medication, the disruption to pig production and flow was deemed comparatively minimal. A medical elimination was designed using cleaning with lime wash and Denagard® (tiamulin hydrogen furarate)[1] in a high/low modality (220ppm for 2 weeks followed by 38 ppm for 2 weeks) to the entire breeding herd. Extensive cleaning and medication continued for 10 weeks in the lactation barns as sows were weaned. Personnel movements during cleaning and medication, reduction in inventory, medication logistics, cleaning logistics and lime wash were planned and implemented.

Results

After the detailed medication and cleaning program was completed, known SD negative gilts were entered and comingled with resident sows. No loose stools were noted or collected in "sentinel" gilts post entry. Two weeks after entry and at two week intervals for a total of 4 collections (30 fecal samples per collection in pools of 5) were randomly collected in late gestation. PCR and anaerobic culture were performed and no samples were detected with *B hyodysenteriae* or *B hampsonii*. Ongoing clinical and diagnostic investigations have not identified either agent after seven months post elimination.

Conclusions and Discussion

Medical elimination costs were estimated at $101,750 (USD) for the elimination plan. An SD infected pig flow cost estimated on a 28 pig/sow/year basis is $700,000 (USD) per annum. The return was estimated to be 1.75 months. With swift identification, evaluation of options and implementation of a solution, this large integrated pig production system has limited and eliminate a costly disease within the production system. This case report exemplifies active surveillance and swift action of the veterinary and production staff to mimimized this costly disease.

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Comparative productive performance of male pigs physically castrated vs immunized with Improvac® in Mexico

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Introduction

Compliance with global standards such as a target market weight, specifications in carcass backfat and loin depth are required in order to fulfill the pork demand in domestic and export markets. Immunization against GnRF is an effective alternative to physical castration of male pigs which can also help producers to achieve production objectives [1,2].

Improvac® (Zoetis) is a vaccine to prevent the presence of boar taint in pig meat from males caused by androstenone and skatole, and now also indicated in some countries for the temporary suppression of ovarian function and estrus in female pigs. The vaccine is approved in 68 global countries, all that have a significant pig production, and with a pre-slaughter withdrawal period of zero days.

The aim of this study was to compare the performance of physically castrated male pigs with entire male pigs immunized with Improvac.

Materials and Methods

A total of 3,821 pigs from 4 production groups from a commercial multi-site system were used: 1,835 physically castrated males (PC) and 1,986 males immunized with Improvac (IC). At 11 weeks of age the pigs were moved from nursery to the finishing facility where they were in 4 barns. The study pigs were housed in 44 pens (20-22 pigs/pen) within each barn according to their body weight. Pigs were marketed according to the farm production system what was fixed to final live weight (≥ 125 kg); pigs were marketed during 4 weeks, from week 12 to week 15 of finishing phase. Animals were categorized as heavy, medium and light pigs based on pen average pig body weight, representing 28, 44 and 28% respectively in each barn.

Pigs in the Improvac group received the first dose when they were 12 weeks old (wo) and the second dose according to the body weight category: at 18 wo (heavy pigs), 19 wo (medium pigs) or 20 wo (light pigs). The diet was the same for both treatment groups and all the study pigs were individually weighed at the beginning and at the end of the study. The average daily feed intake ADFI was measured using the BinTrac® system. Endpoints included average daily gain (ADG), feed conversion rate (FCR), feed efficiency (FE), mortality, cull rate and days in fattening (days in). Data were analyzed using a randomized complete block design (barns).

Results

Immunologically castrated pigs had significantly better ADG (1.04 vs 1.01 kg/d,) and FE (0.422 vs 0.394) than physically castrates (P<0.05) (Table 1). PC pigs had higher ADFI (2.49 vs 2.39 kg/d) and FCR (2.54 vs 2.37) than IC pigs (P<0.05) (Table 1). The average final body weight tended (P=0.08) to be higher for IC pigs (Table 1). The days in mortality and cull rates were not affected (P>0.05) by the castration method (Table 1).

Table 1. Pig performance during finishing phase using physical or immunological castration.

Variable	Treatment		SEM	P
	PC	IC		
Pigs per barn, n	459.00	496.00	1.920	0.001
Initial body wt, kg	32.52	32.53	0.303	0.98
Mortality, %	3.92	3.68	0.200	0.45
Culls, %	3.47	3.67	0.479	0.79
Final body wt, kg	125.12	128.60	0.610	0.08
Days in, d	91.57	92.47	0.369	0.18
ADG, kg	1.01[a]	1.04[b]	0.004	0.01
ADFI, kg	2.49[a]	2.39[b]	0.006	0.001
Feed conversion	2.54[a]	2.37[b]	0.018	0.008
Feed efficiency	0.394[a]	0.422[b]	0.003	0.006

¶ Least square means, Data was analyzed using a randomized complete block design (barns). SEM: standard error of the mean. [ab] Different letters in the same row mean figures are statistically different (P<0.05).

Conclusions and Discussion

There are important production benefits for pigs immunized with Improvac vs. pigs physically castrated. IC pigs had better ADG and FCR. Grading is a common strategy to improve FCR and weight uniformity. This trial was designed to take adavange of immunization combined with grading, to our knowledge the first time is done under commercial conditions, improving pig performance even when the pigs are marketed following fixed final weight management.

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Weekly performance of immunocastrated boars and gilts in *ad libitum* feeding

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Introduction

The immunocastration deactivate testicular functions by neutralization of the hormones of the hypothalamic pituitary gonadal axis (4). It is an attractive alternative to surgical castration, as it efficiently reduces boar taint produced by skatole and androsterone (1, 2, 3, 4). The aim of this study is to investigate the effect of the immunocastration on the performance of growing pigs in *ad libitum* feeding throughout the finals finishing weeks.

Materials and Methods

The experiment was carried out in a commercial grow to finish barn in Santa Catarina state, Brazil. Entire male pigs were housed and subjected to two doses of the Improvac® (Zoetis) anti-GnRH vaccine - the first and second injection with an average of 40 and 80 days on feed respectively, 25 days before slaughter. Gilts were not vaccinated.

Pigs were allotted in 24 pens of 50 pigs each, in three replications. A total of 1,784 immunocastrated boars and 1,794 gilts were stocked at *ad libitum* feeding and weekly weighed thought out the growing to finishing period.

A repeated measure design in a mixed model approach was used to access the main effects of sex and days on feed. The performance traits were average daily gain (ADG), average daily feed intake (ADFI) and feed conversion (FCR=ADFI/ADG). Different variance-covariance matrix was tested using the smallest Schwarz's Bayesian criterion to choose the best fit model (SAS® University Edition, Inc., Cary, NC). Differences with *P*-values less than 0.05 was considered as statistically different and less than 0.10 was considered as tendency.

Results

There were no significant differences between boar and gilts in all traits evaluated before second injection of Improvac® (p>0.05). Afterwards, at 80 to 87 days on feed, there was a significant increase in ADFI of the boar compared to gilts (3.203 *vs* 3.082 kg/day, p<0.001), as there was in ADG (1.476 *vs* 1.336 kg/day, p<0.0001). All phases, from 80 days on feed, immunocastrated boars had higher ADFI and ADG than gilts (table 1).

Through the time, the immunocastred boars had its maximum ADG between 87 to 101 days on feed, 7 to 15 days after second injection of Improvac®, as it happened at day 80 to 94 for gilts. Moreover, there were no differences in FCR from 66 to 94 days on feed, 15 days around the immunocastration, as it happened for gilts. In ADFI, there were no differences on the two weeks before immunocastration as it improved weekly afterwards. In gilts, the ADFI has its plateau after 80 days on feed.

Conclusions

There were no main differences in performance between boar and gilts before the second injection of Improvac®. Throughout 15 days after second dose, immunocastred boar kept their best FCR even though there were significant increase of ADFI. Gilts had a very flat FCR until the 94 day on feed having its growth plateau at 80 to 94 days on feed.

Table 1. Comparative performance between immunocastred boars and gilts in *ad libitum* feeding.

Item[1]	DOF[2]	Gender		SEM[5]	*P*-value[6]	
		Boars[3]	Gilts[4]		Gender	DOF
ADFI, kg/day	66-73	2.874^{a}	2.875$_{a}$	0.032	0.56	<.001
	73-80	2.850^{a}	2.872$_{a}$	0.032	0.18	0.55
	80-87	3.203^{b}	3.082$_{b}$	0.032	<0.001	<.001
	87-94	3.329^{c}	3.063$_{b}$	0.032	<.0001	<.001
	94-101	3.716^{d}	3.010$_{b}$	0.032	<.0001	<.001
	101-106	3.952^{e}	3.099$_{c}$	0.032	<.0001	0.001
	106-113	3.693^{d}	3.044$_{bc}$	0.033	<.0001	<.001
ADG, kg/day	66-73	1.265^{a}	1.256$_{a}$	0.017	0.70	<.001
	73-80	1.254^{a}	1.215$_{ac}$	0.017	0.20	0.56
	80-87	1.476^{b}	1.336$_{b}$	0.017	<.0001	<.001
	87-94	1.533^{c}	1.304$_{b}$	0.017	<.0001	0.08
	94-101	1.463^{bc}	1.163$_{ac}$	0.017	<.0001	<.001
	101-106	1.346^{ae}	1.037$_{d}$	0.017	<.0001	<.001
	106-113	1.010^{f}	0.850$_{e}$	0.019	<.0001	<.001
FCR	66-73	2.272^{a}	2.289$_{a}$	0.042	0.62	0.28
	73-80	2.272^{a}	2.363$_{a}$	0.042	0.19	0.70
	80-87	2.173^{a}	2.309$_{a}$	0.042	0.13	0.18
	87-94	2.176^{a}	2.349$_{a}$	0.042	0.05	0.57
	94-101	2.541^{b}	2.589$_{b}$	0.042	0.09	<.001
	101-106	2.936^{c}	2.991$_{c}$	0.042	0.42	<.001
	106-113	3.663^{d}	3.576$_{d}$	0.048	0.99	<.001

[1]ADFI: Average daily feed intake. ADG: Average daily gain. FCR: Feed conversion rate. [2]DOF: Days on feed. [3]Different superscript letters within boar column differ throughout DOF at p<0.05. [4]Different subscript letters within gilts column differ throughout DOF at p<0.05. [5]SEM: Standard error of the mean between gender. [6]*P*-value from a t-test of a repeated measured mixed model design with an autoregressive variance-covariance matrix.

Acknowledgments

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Use of oxytocin in farrowing: effects on piglet mortality

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Introduction

Piglet stillbirth is a multifactorial issue and frequently associated to improper use of oxytocin, dystocia, farrowing duration, sow body condition and labor quality (3, 5). Oxytocin is frequently used on swine farms to decrease farrowing time and birth interval as an auxiliary method to prevent stillbirths. However, recent studies have shown that oxytocin overuse or when used too early in the birth process, can increase the number of pigs stillborn by causing rupture of umbilical cords and compromise oxygen supply to piglets during the birth process (2, 3, 4). The objective of the present study was to evaluate the effect of oxytocin usage during farrowing in stillbirth rate.

Material and Methods

A total of 138 litters from an 1,100 purebred pig farm were evaluated. Farrowing room, parity order, farrowing type (normal or induced by prostaglandin), gestation length, farrowing duration, total born and use of oxytocin during parturition were recorded for each sow. Stillbirths piglets were necropsied and classified in prepartum (PR), intrapartum (IP) or postpartum (PP) stillbirths according to skin color and eyes characteristics, umbilical cord hemorrhage and presence of meconium staining (1). All data recorded were analyzed using MIXED model adjusted to Tukey-Krammer test ($P< 0.05$) with multiple comparisons using the LSMEANS in SAS® University Software (SAS® University Edition, Inc., Cary, NC).

Results

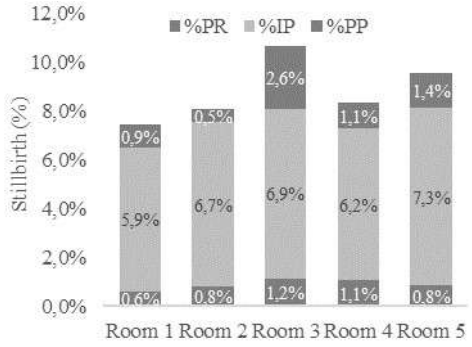
The average found in the number of total born was 16.53. Oxytocin was used in 18.7% of farrowing sows. The use of oxytocin significantly increases the rate of stillbirths, presenting a significant effect ($p <0.05$) for IP and being suggestive for PP (Table 01). The difference in the classification of stillborn piglets between the delivery rooms suggested an effect on the quality of labor during delivery (Graph 01).

Conclusions and Discussions

Inappropriate use of oxytocin increase the numbers of stillbirths. Oxytocin should not be used as the single practice to aid farrowing or as a substitute for obstetrical assistance. Farrowing attendants should be able to identify the right time to carry out obstetric interventions.

Table 01: Effect of oxytocin in stillbirth rate.

Use of oxytocin and stillbirths %				
Stillbirths	**Oxytocin**		**SEM**	***P*-value**
	Yes	**No**		
prepartum	0,4%a	0,1%a	0.06	0.93
intrapartum	9,1%a	6,3%b	0.19	0.02
postpartum	1,5%a	0,7%a	0.07	0.06



Graph 01: Stillbirth piglets classification in prepartum (PR), intrapartum (IP) and postpartum (PP).

Acknowledgements

Acadrolli, Agroceres PIC and UFMS

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Detection of APX-toxins I, III and IV by PCR by the use of cotton ropes

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Introduction

Herd screening for specific pathogens can be labor intensive and burdensome for humans and animals. Rope sampling is an easy and gentle method that is successfully used for the detection of antibodies or genome fragments for PRRSV, SIV, PCV2 (1) or others (2). The present examination was conducted to evaluate the suitability of rope sampling for the detection of *Actinobacillus pleuropneumoniae*-infections in fattening pig farms.

Materials and Methods

The study took place in two APP-positive fattening farms located in North-Western Germany. Farm A vaccinated the animals against APP (Porcilis® APP, MSD Animal Health) at the age of eight and twelve weeks. Farm B did not vaccinate against APP. On farm A 280 pigs allocated to eight pens (35 pigs / pen; 2 ropes / pen) and on farm B 215 pigs allocated to eight pens (27 pigs / pen, 2 ropes / pen) were included. Ropes were placed directly after arrival of the animals (12 weeks of age, T0) and then daily for six consecutive days. Afterwards sampling took place every second week until slaughter (week three (W3) until week 15 (W15)). Oral fluid was examined by PCR (BioChek APP q-PCR test kit).

Results

Genome fragments of APP were detectable from the first day on (T0). APX4- and APX3-toxin was detectable in both farms, whereas APX1-toxin was only present on farm A (figure 1-3).

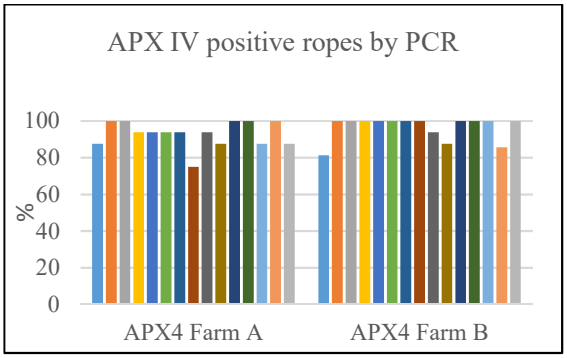

Figure 1: Percentage of APXIV-toxin positive ropes on farm A and B. Each column represents a sampling (T0-W15).

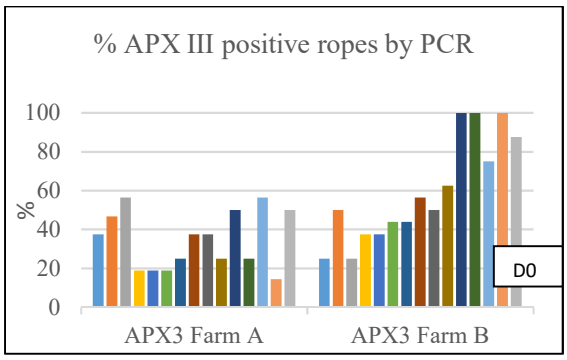

Figure 2: Percentage of APXIII-toxin positive ropes on farm A and B. Each column represents a sampling (T0-W15).

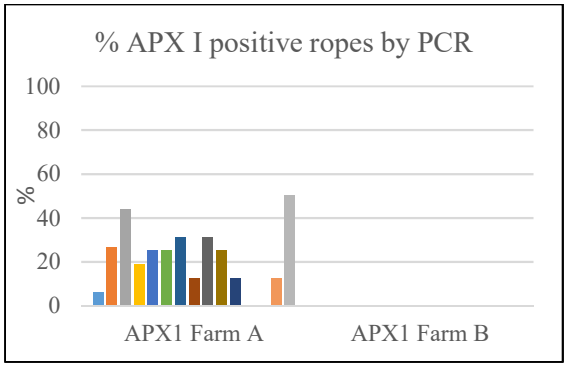

Figure 3: Percentage of APXI-toxin positive ropes on farm A and B. Each column represents a sampling (T0-W15).

Conclusions and Discussion

In both farms the APP-infection could be confirmed directly after placement of the pigs in the fattening unit. Although different APX-toxins are not produced solely by APP, the use of the toxin-PCR might be helpful to determine the virulence of present APP-strains on the farms reflecting the need of vaccination against APP in farm A.

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AMR – Anti-microbial reduction - Periodic risk management system structuring the usage of antibiotics

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Introduction

It is well know the definite strategy on the field of anti-microbial resistance: reducing the number of resistant bacterias – and it's important corner stone: antibiotics usage in animals. Unfortunately, authorities have lack of knowledge about the actually antibiotics given to the livestock by farm/stable, furthermore they have no information about how much antibiotics were given to the dedicated animals, where do they come from, who bought them from whom, when and why. However, this information is required to fulfill their obligations to reduce unnecessary use of antibiotics. Finally, from food safety point of view it is important to know the residues of microbials per one kilogram of livestock product and through that residues of microbials per food unit. This study introduces a new way for the monitoring of antimicrobial use on farms and evaluation of the results.

Materials and Methods

Within in a newly set up antimicrobial reduction system (AMR) users have to create a virtual farm – on which they can follow up what happened on their real livestock farm – and they just have to register a few daily input data (values): purchase, sales, mortality – all in piece/weight – and treatments. From these values and the data given at the implementation phase the software is able to calculate the feeding curve (days, feed consumption, ADG) by which the program knows how "big/what weight" that pig should be and provides the data to the register. The AMR – with the help of its master data filled by the user – knows the maximum usage rate (doses) for every medicine for every type and "weight/size". AMR has a documentation in which users can store and monitor all of their antimicrobial susceptibility (resistance) tests which is a validation point in the system. Furthermore, the system knows all types of antibiotics and medicines (registered by authorities) and all distributors and suppliers details.

Results

Firstly, we can order the antibiotics directly by use of e-recipe sealed and signed by vets electronically and immediately – this is another validation point in the system. It means that the procurement is automated so as the inventory recording and the treatment log. Therefore, we will know every stock on every farm, thus the authorities, that get access to the database, can tackle the sales of antibiotics and/or medicines. The AMR also calculates the necessary amount of medicines and antibiotics if the data are entered. Secondly, we know the usage of all registered medicines, hence, the sales of the suppliers and distributors can be reported. Thirdly, all users know the antibiotics use by production unit and know their animal health status evaluation. Lastly, users can also include feed additives into the system, not just antibiotics.

Future Opportunities

Using of data provided for AMR we have the opportunity to create an analysis software that can be part of the One Health Concept systems by creating reports on a national (memberstate) and/or EU basis which are suitable for risk analysis. This software can generate a structured database which can be useful for the authorities, as well. Furthermore, we could include minimum inhibitory concentration (MIC) values into the system, thus the authorities and the farmers would be able to monitor the possible antibiotic resistances.

Conclusions and Discussion

By this new approach and e-tool we are able to evaluate the use of antibiotics by calculating the used antibiotics per one kilogram of animal origin food by livestock farms, countries or region. Such system as AMR – as an integrated data collecting platform – could be an essential part of food-chain safety platform.

Circo/MycoGard® field efficacy assessment in a PCV2-sow-vaccinated farm in Mexico

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Introduction

Circo/MycoGard® is a PCV2b/Mhp combination vaccine with high safety, antigen purity and cell-mediated immunity due to the triple adjuvant system that it contains. We assessed the field efficacy of 1 dose of Circo/MycoGard® in comparison with 2 doses of Circumvent® PCV M in a PCV2-sow-vaccinated farrow-to-finish farm in Mexico.

Materials and Methods

A 300-sow farrow-to-finish farm that was PCV2, Mhp and IAV positive, and PRRSV and PEDV negative was selected. Sows were routinely PCV2-vaccinated at weaning (pre-breeding) with a 2-mL dose of Circumvent® PCV M. On each week during 3 weeks, all available piglets were randomly enrolled in 2 treatment groups. A total of 304 pigs were enrolled (~100 pigs/week and ~150 pigs/treatment group). Pigs were blocked by litter and weight at enrollment/weaning, so both treatment groups were equally represented in each litter.

At weaning/enrollment (~18 days of age), pigs were vaccinated with either Circo/MycoGard® (2mL) or Circumvent® PCV M (1mL) as a first dose. At 2 weeks after weaning, Circo/MycoGard® vaccinated pigs received 1 mL of Saline and Circumvent® PCV M received a second dose of 2 mL. Pigs were ear-tagged and weighed at enrollment/weaning, end of nursey and at marketing. In each nursery and finishing pen, approximately the same number of pigs per treatment group were randomly allocated. Performance was adjusted for start weight and cohort effects as well as to 56, 76 and 132 days of feed for nursery, finishing and wean-to-market phases. Data was analyzed using generalized linear regression models in R statistical software[1].

Results

Livability and ADG of pigs vaccinated with 1 dose of Circo/MycoGard® at weaning was equivalent to 2 doses of Circumvent® PCV M in a farrow-to-finish farm with a PCV2 sow vaccination program. Table 1 summarizes the performance of the 2 vaccinated groups.

Conclusions and discussion

One dose of Circo/MycoGard® performed similarly to 2 doses of Circumvent® PCV M in farrow-to-finish farm with a PCV2 sow vaccination program. Even though we did not conduct regular testing for PCV2 in either sows or pigs, our results suggest that in farms were PCV2 sow vaccination programs, PCV2 vaccine programs of dose at weaning can be as effective as 2 doses programs likely because of the reduction of PCV2 infections in both sows and pigs.

References:

1. R Core Team (2020). R: A language and environment for statistical computing. Vienna, Austria, R Foundation for Statistical Computing: Statistical Software.

Table 1. Circo/MycoGard® field efficacy assessment using a flow-based natural infection approach

Indicators	Circo/MycoGard® 1 dose	Circumvent® PCV-M 2 doses	p-value
No. pigs	112	116	-
No. deaths and removals	2	8	-
Wean-to-market Livability, %	98.4	93.7	0.05
Weaning weight, kg	5.6	5.6	-
Nursery weight, kg	30.8	29.6	-
Market weight, kg	101.5	100.3	-
Nursery ADG, g/day	448	428	0.03
Finishing ADG, g/day	923	939	0.92
Wean-to-market ADG, g/day	724	716	0.44

Circo/MycoGard® field efficacy assessment using a flow-based natural infection approach

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Introduction

Circo/MycoGard® is a PCV2b/Mhp combination vaccine with high safety, antigen purity and cell-mediated immunity due to the triple adjuvant system that it contains. We assessed the field efficacy of Circo/MycoGard® using a flow-based approach under natural field conditions and infections in several batches within one flow of production.

Materials and Methods

A sow farm of ~1,200 sows located in Ontario and known to be positive for Mhp and PCV2, and negative for PRRSV and PEDV was selected as the pig source. Within this flow, one nursery site with one barn that has 3 rooms was selected. Each room was filled within 4 days with ~600 pigs and about every two weeks. Feeder pigs were moved to the finishing site that has 3 barns. Each finishing barn has 2 rooms that were filled with the ~600 pigs coming from each nursery room. A total of 10 batches were evaluated over time and every other batch was assigned to each vaccine (Circo/MycoGard® and Fostera® PCV MH). Pigs were weaned at ~21 days of age and vaccinated at ~10 days after weaning with one single dose of either vaccine. Mortality, ADG, live weight/ton feed and gross margin/ton feed from each batch were adjusted to 52, 123 and 175 days on feed for nursery, finishing and wean-to-finish phases respectively. FCR was adjusted to 55, 50-250 and 290 lb of live weight for each phase respectively. For the gross margin/ton feed estimation, feed cost per ton was 364 CAD and price per lb of live weight was 0.81 CAD. Nutrition and diets were considered similar amongst batches. Data was analyzed using Wilcoxon rank sum test and generalized linear models in R statistical software[1].

Results

Performance was similar between Circo/MycoGard® and Fostera® PCV MH in all production phases. Adjusted gross margin and live weight per ton of feed were similar between Circo/MycoGard® and Fostera® PCV MH as detailed in Table 1.

Conclusions and discussion

Circo/MycoGard® performed similarly to Fostera® PCV MH. Even though seasonality, and PCV2 and Mhp infection dynamics over time were not assessed in those groups, our performance results suggest that well-designed flow-based studies can be a powerful tool for adequate field economic and production evaluation of PCV2 vaccines.

References

1. R Core Team (2020). R: A language and environment for statistical computing. Vienna, Austria, R Foundation for Statistical Computing: Statistical Software.

Table 1. Circo/MycoGard® field efficacy assessment using a flow-based natural infection approach

Phase	Adjusted closeout parameters	Circo/MycoGard® (Mean [Range])	Fostera® PCV MH (Mean [Range])	Wilcox p-value
Nursery (52 days)	No. pigs	2929	3102	-
	No. closeouts	5	5	-
	Mortality (%)	3.9 [2.2-5.0]	2.9 [1.0-4.8]	0.42
	ADG (lb/day)	1.03 [0.97-1.08]	1.00 [0.88-1.11]	0.55
	FCR	1.53 [1.48-1.57]	1.57 [1.45-1.68]	0.31
	Live weight (lb)/ton feed	1862 [1754-2041]	1767 [1420-2010]	0.84
Finishing (123 days)	Mortality (%)	3.9 [3.7-4.4]	3.6 [2.9-4.4]	0.31
	ADG (lb/day)	1.99 [1.83-2.15]	1.98 [1.86-2.08]	0.84
	FCR	2.65 [2.49-2.80]	2.67 [2.50-2.77]	0.69
	Live weight (lb)/ton feed	1107 [1050-1194]	1105 [1032-1240]	1.00
Wean-to-Finish (175 days)	Mortality (%)	7.7 [6.1-9.2]	6.5 [4.1-9.2]	0.69
	ADG (lb/day)	1.70 [1.60-1.78]	1.68 [1.59-1.79]	0.84
	FCR	2.69 [2.53-2.82]	2.71 [2.60-2.82]	0.84
	Live weight (lb)/ton feed	875 [833-902]	869 [838-895]	0.69
	Gross margin (CAD)/ton feed	345 [311-366]	340 [315-361]	0.69

A data analytics model for assessing the vulnerability of contagious of African Swine Fever in Colombia

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Introduction

African Swine Fever (ASF) is a highly contagious and deadly hemorrhagic disease affecting pigs, with devastating consequences for economies of infected countries (1,2,3). Several countries are free of ASF, including Colombia. Nevertheless, because of its transboundary nature and the lack of vaccines, the thread of contagion in most free countries is considerably high (4). Vulnerability assessment of disease contagion aims to establish levels of risk of contagious of this disease quantitatively (5). This surveillance strategy is fundamental for devising cost/effective epidemiological vigilance strategies (1,2,3). However, the levels of vulnerability to ASF depend on local, highly complex multifactorial environmental, ecological, and social factors, which can be challenging to identify and quantify even for domain experts. In this work, we introduce a novel data analytics-based model aimed to quantify the ASF levels of risk of contagious, for scenarios when the disease is not present yet.

Materials and Methods

A systematic review based on the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) method was conducted in order to identified risk factor associated with transmision of ASF. An indicator-based model provided the vulnerability quantification (5). For this, first, a systematic review guided by expert criteria provided a set of critical factors of vulnerability for Colombia (6). Broad categories of vulnerability grouped these factors. Quantification of each factor was performed by using multiple data sources describing different aspects of ASF, including biosecurity surveys, animal mobilization, and productive process descriptions, and among others. Specific mathematical models were devised to compute a normalized 0-1 index of vulnerability per municipality - per factor. These indices were linearly mixed per municipality by using a convex combination. Weights for these combinations were defined by 14 Colombian domain experts using linguistic quantifies and ordered weighted average operators (7). A similar strategy was used to combine the resulting categories. Finally, a visualization tool was developed to explore the vulnerability factors.

Results

A total of 168 risk factors were identified in 2364 articles screened throgouth PRISMA method, 22 risk factor were adjusted for experts in the pig production system in Colombia. Table 1 reports 22 risk factors and the seven broad categories of vulnerability, together with the corresponding relevance weights obtained from the experts.

Table 1. Categories and factors of vulnerability for ASF with their corresponding weigths used for linear combination .

Category	Category weight	Factor	Factor weight
Biosecurity	0.22	Feeding practices	0.21
		Infrastructure and use of facilities	0.19
		Indirect contact by staff	0.18
		Disposal of dead pigs	0.17
		Borrow boar	0.14
		Mixed crop-livestock productions systems	0.12
Movement	0.2	Pig movement	0.55
		Pork products movement	0.45
Biophysical space	0.17	Proximity to international borders	0.14
		Proximity to international ports and airports	0.13
		Proximity between farms (Density)	0.12
		Proximity to roads	0.12
		Proximity to dumps	0.11
		Proximity to towns	0.10
		Proximity to animal markets	0.10
		Proximity to slaughterhouses	0.09
		Proximity to food processing plants	0.09
Environment	0.15	Wild swine presence	1.00
Pig Productive System	0.14	Pig farm orientation	0.54
		Intensity of pig production	0.46
Socioeconomic sorrounding	0.13	Entry of live pigs, meat and meat products	0.58
		International movement of people	0.42

Figure 1 shows the user interface devised to explore different vulnerability factors. As observed, different categories ("categorias") and vulnerability factors ("factores de riesgo") were quantified in different risk levels for the region of Colombia.

Figure 1. User interface for the vulnerability of ASF contagious. Different factors and categories can be explored by using the tool.

Conclusions and discussion

Implementation of successful epidemiological surveillance strategies requires objective evidence for cost/effective resource allocation. In the case of ASF free areas, where probably there is no available information on previous contagious, models based on data may provide these quantifications. Our results show that a combination of evidence coming from existing literature, mathematical modeling and expert knowledge may provide quantifications of vulnerability for ASF.

Acknowledgments

This work was funded by PorkColombia - Fondo Nacional de Porcicultura.

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African Swine Fever spread throught animal movement in Colombia using the Pig Mobilization Network

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Introduction
ASF is one of the most devastating diseases of swine because its significant sanitary, socioeconomic consequences and great impact to agriculture (1,4). In this study, the information of the swine mobilization routes and Suceptible-Infected (SI) model (2,3) was used to evaluate the potential evolution of the African Swine Fever (ASF) spread in Colombia.

Materials and Methods
A systematic review based on the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) method was conducted in order to identified ASF spread models. The proposed ASF spread model through direct contacts is shown in Algorithm 1.

Algorithm 1. ASF spread model

ASF spreed($\beta, \lambda, d_0, I_0, G$)
1: $t = 0$
2: $G_t = G[startDate]$
4: $towns_t^I = \{I_0\}$
3: **while** ($t < \lambda$) **do**
4: $towns_{t+1}^I = \{I_0\}$
5: **for each** $i \in towns_t^I$ **do**
6: $subG_t^i$ = sample(G_t^i)
7: **if** $subG_t^i$ **is not** { } **then**
8: N_t^i = neighborhood($i, subG_t^i$)
9: **for each** $n \in N_t^i$ **do**
10: w = getPigs(i, n)
11: $isInfected$ = SI-model(w, β)
12: **if** $isInfected$ **then**
13: $towns_{t+1}^I = towns_t^I \cup n$
14: $t = t + 1$
15: $G_t = G[d_0 + t]$
17: **return** $towns_t^I$

The initial parameters for the ASF spread model are: the infected rate β, the simulated days λ, the initial date for the simulation d_0, the initial infected town I_0, and the mobilization network G.
After the initial infection, the transmission processes occur throughout the study region (line 5-13) considering the parameterization of the model[1]. The model assums that if a pig in the mobilization becomes "infected" (line 11), the town where that mobilization was heading becomes "Infectous" (line 13).

Results
A total of 1000 different epidemics was run over different scenarios of initial date d_0 i.e., January 01, April 01, July 01, and October 01. The other parameters for the ASF spread model β, λ, I_0, and G were 0.001, 30 days, Ebéjico-Antioquia, and 2015 swine mobilization, respecively.
Over the 1000 epidemics for the four experiments, the probability ($P(i_\lambda^I|I_0)$) was calculated, see Figure 1. In general, the ASF simulations produced small and short epidemics, and are higly dependent on the time and quantity of animals mobilized.

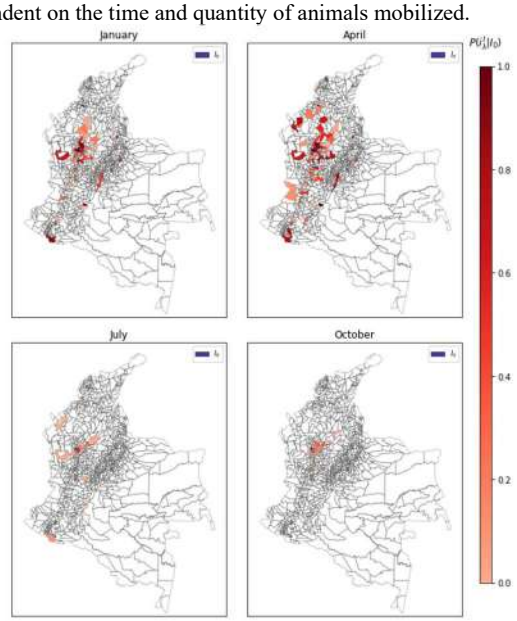

Figure 1. Probability of the i town get "Infectious" at the end of simulation (i_λ^I) given that the spread begins at I_0.

Conclusions
The study presented here is one of the first to describe quantitatively the potential spread of ASF from different towns in Colombia. Simulation studies conducted here were useful for estimating the potential risk of introduction or spread of ASF over specific initial infected town throught assessing the main routes of spread transmission.

Acknowledgments
This work was funded by PorkColombia - Fondo Nacional de Porcicultura.

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[1] A set of random mobilizations is chosen from the total monthly mobilizations to transform montly to daily movements (line 6).

Evaluation of an audio-based sensor platform to assess directionality and classify patterns of clinical respiratory episodes in large growing pig populations

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Introduction

Audio-based sensor systems hold the potential to remotely differentiate the primary etiology of clinical episodes of respiratory disease. The purpose of this project was to evaluate the ability of an audio-based sensor system to detect the directionality of respiratory episodes, and to classify detected patterns of clinical respiratory disease in growing pigs according to their primary etiology under large-scale commercial production conditions.

Materials and Methods

Audio sensor devices (SoundTalks, Leuven Belgium) were installed in three large commercial wean-to-finish facilities designed to house 1200 to 2400 pigs per airspace. An algorithm-based respiratory distress index (RDI) was continuously generated from recorded sound files and uploaded to a cloud database. The data were charted and patterns of cough were categorized. For each RDI episode, diagnostic samples were collected and tested by PCR for PRRS virus, IAV-S virus, *Mycoplasma hyopneumoniae*, PCV2 virus and *parainfluenza*. Episodes were aligned with their corresponding diagnostic results and the resulting aggregate cough patterns were characterized.

Results and Discussion

The ability of the multiple device configuration to determine directionality of RDI episodes was assessed. The directionality of the onset and progression of the IAV-S (H1N1) and *Mycoplasma hyopneumoniae* episodes throughout the 2400 head barn were evident in the data (Figure 1).

Each device represents an 18-20 meter diameter sound detection "zone". Within the footprint of an airspace, the detection and directionality of cough is then a function of the square meters covered by the "zones" out of the total possible square meters in that contiguous airspace. As such, the detection, directionally and movement (ebb and flow) of RDI episodes through the airspace are made possible, enabling better characterization and understanding of respiratory disease behavior

RDI episodes were detected across the three farm sites, including: IAV-S (H1N1), IAV-S (H3N2), and *Mycoplasma hyopneumoniae* (Figure 2). Diagnostic testing of oral fluid samples and laryngeal swabs by PCR and sequencing confirmed the presence of IAV-S (H1N1), IAV-S (H3N2) and *Mycoplasma hyopneumoniae* for the three RDI episodes, respectively. PRRS PCR testing results were negative for all samples collected at all three RDI episodes.

Two distinctive RDI patterns were detected across the three farm sites, one associated with IAV-S (H1N1 or H3N2), and another associated with *Mycoplasma hyopneumoniae*. IAV-S associated RDI patterns had a distinctive bi-modal shape, whereas the pattern associated with *Mycoplasma hyopneumoniae* showed a gradual relatively linear rising pattern. The detection of the respiratory disease episodes by the audio sensors ranged from an estimated 2-5 days earlier than detection by farm personnel.

Figure 1: Example of the ability of a configuration of multiple audio sensor devices to determine spatial directionality of the onset of cough episodes.

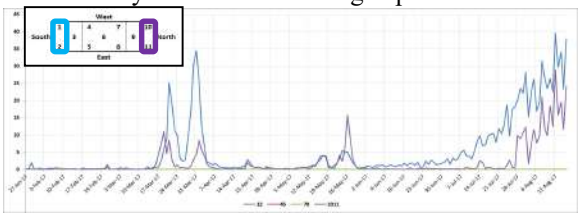
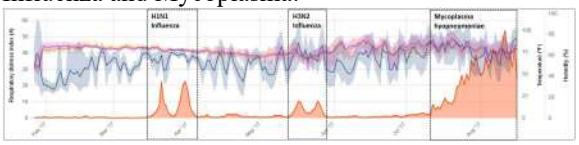


Figure 2: Example of a Respiratory Disease Index (RDI) chart with temperature and humidity data from a 2400 head wean-to-finish barn experiencing clinical episodes of Influenza and Mycoplasma.



Conclusions

Monitoring the health and performance of growing pigs by using audio-based devices and sensors to capture, quantify, assess directionality of and categorize sound events such as coughing can enable earlier and more precise detection of relevant clinical episodes, and result in more timely and targeted intervention. In doing so, the impact of respiratory disease on animal welfare and performance can be better minimized and the related financial impact can be better reduced, enabling optimization of profitability.

With this information, local site managers can better adjust and respond with more timely, appropriate diagnostics and treatment. Further, those responsible for flows/systems and areas/networks can better assess larger scale behavior of specific disease agents and the clinical impact of intervention and control protocols.

Evaluation of a technology platform to measure near real-time movement of animals, assets and personnel in a pig production network in the United States

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Introduction

The pig and pork production industry is highly networked and mobile – with various types of movements occurring numerous times per day within farms, between farms, across production systems and throughout production networks. Production systems experience movements of pigs, semen, feed, supplies, assets and personnel – within farm sites, between farm sites, and among non-production sites (e.g., feed mills, truck washes, offices, warehouses). All movements inherently carry with them varying levels of disease introduction and transmission risk by animals, people and/or fomites within and among farm sites, with often serious consequences on animal productivity and business performance.

The objective of this project was to design, develop and evaluate an integrated measurement system to capture movement records of personnel and assets within and among sites to enable the more objective assessment of movement-related risks of disease introduction and transmission.

Materials and Methods

A large multi-farm system and production network in the United States was enrolled in the project. The system is composed of six large sow farms and 56 grower sites. All semen is sourced from an external boar stud. The system has an owned feed mill.

At each animal production site, zones were defined, and location beacons were assigned to and placed within each zone. For live animal housing space, each zone typically represents an entire barn or an airspace for breeding, gestation and farrowing. For other areas of the farm, defined zones represented specific rooms and work areas (e.g., Medication Room, Tool Room, Pressure Washer Room, Sow Wash area, Load out chute).

Location beacons were installed within each zone, and were set to transmit 20 meter radius signal. The number of location beacons placed in each zone varied, depending on the length x width dimensions of the zone.

Significant assets (e.g., trucks, trailers, feed carts, robots, power washers, semen coolers) were tagged with asset beacons. Asset beacons were set to transmit a signal to a radius of five meters.

A risk level (0-10) was assigned to each zone-to-zone movement pair. The assigned risk level was selected based on the estimated risk of movements between two different zones related to their respective zone risk characteristics (e.g., site type, stage of production, animal age, known disease status, production system network connection status).

Results and Discussion

In Figure 1, left image, the arrows point from source to destination with number of movements (color of arrow corresponds to risk level). In the right image, movement volume for the defined interval are represented as a heat map of origin and destination movement intensity (color of zone corresponds to number of movements in period being displayed).

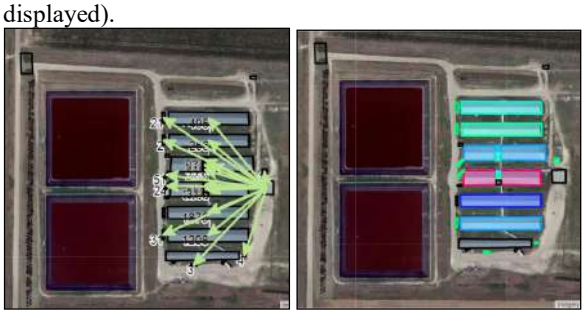

Figure 1: Examples of personnel and asset movements over a 31 day period in Sow Farm A displayed on satellite map

In Figure 2, the number and risk level of all pairs of origin-destination movements for personnel and assets are displayed in a bubble chart format.

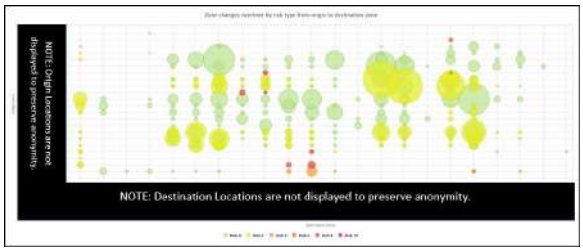

Figure 2: Scatter plot of Origin location (Y-axis) to Destination location (X-axis) movements over a 31 day period in Sow Farm A (Note: X and Y axis zone name labels are hidden to protect anonymity)

Conclusions

This project is an evaluation of the feasibility of utilizing a Bluetooth Low Energy (BLE) based technology in a system-scale implementation in pig production. This technology can be used as a means for the recording of personnel and asset movements, enabling an improved understanding of disease introduction and circulation risks within and among sites across production networks in near real-time. As this and any additional implementations progress, opportunities are expected to arise for assessing the platforms investigative contribution towards understanding the source(s) of disease agent introduction and continued circulation within and between farms.

REPRODUCTION

Influence of polyphenols (hydroxytyrosol and carnosic acid) supplementation on reproductive performance of sows

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Introduction

Swine production has markedly increased its reproductive performance over the past 50 years. One of the most important limitations to continue increasing the size of the litter, or to fully express the genetic potential of the breeders, is the prenatal mortality of embryos. Among other causes the antioxidant/oxidative balance has a key role (1,2,3). Antioxidants are used as molecules that slow down the redox process boosted by free radicals, ending oxidative stress (4) which adversely affects prenatal development, alternative sources of antioxidants are polyphenols and, particularly, the current study aimed to analyze the usefulness of hydroxytyrosol and carnosic acid during gestation supplementation in the reproductive performance of sows in commercial herds.

Materials and Methods

A total of 95 gilts ans sows (1-7 parities) manteined under the same conditions, were sorted into two treatment groups during the whole gestation period to compare the effects of supplementation in the feed with hydroxytyrosol and carnosic acid (MiaPhenol; 150 g/kg feed; total phenol content 35 mg/g MiaPhenol; group MPH), remaining a control group untreated (CG). Both groups received the same basal diet formulated to meet the requirements during pregnancy, feeded once a day, while having ad libitum access to water. The daily amount of feed supplemented during the entire gestation phase was 2.5 kg per animal and day. Data were collected individually for total number of born piglets and their live weight, alive-born piglets, stillborn piglets and mummified piglets at the farrowing moment (<12 h post farrowing). Data were analyzed using a statistical package SPSS v. 15.0. Comparisons of means were made using a Student's t-test. Significant differences of less than 0.05 were always considered significant.

Results

Throughout the treatment, the group MPH showed higher number of total born piglets per litter (18.32 vs. 16.39; P=0.02), as well as, number of live-born piglets per litter (16.66 vs. 14.78; P=0.04) compared to control group. Number of stillborn and mummified piglets per litter did not significant differs among groups (1.41 vs. 1.23; P=0.59; 0.23 vs. 0.37; P=0.25 respectively). The live weight at birth of the piglets was exactly the same for both treatments (1.35 vs. 1.35 Kg; P=0.96). Nevertheless, the total litter live weight differed significantly in favor of the MPH group (22.14 vs. 17.27 Kg; P<0.0001). Besides, the birth live weight coefficient of variation is shown lower in the MPH group (15.10% vs. 19.11%)

Conclusions and Discussion

The present trial indicates that the supplementation with hydroxytyrosol and carnosic acid during gestation period improve significantly both number of total born piglets and alive-born piglets. Interestingly, the mean weight of piglets at born was the same, but the difference in the number of piglets resulted in an important difference for the total litter weight per farrow. However, the addition of polyphenols do not affect stillborn and mummified number of piglets per litter.

Furthermore, the piglets birth live weight coefficient of variation as a measure of relative interpretation of the degree of variability, was positively decrease when antioxidants were supplemented. So, this trial suggests that the addition of polyphenols could results in increased number of piglets, increased total weight of litter and reduction in weight variability.
Further studies are needed to clarify mode of action and efficacy of MiaPhenol.

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Reproductive performance of gilts submitted to post-cervical artificial insemination using 1.5 or 2.5 billion sperm cells in the inseminating dose

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Introduction

Post-cervical artificial insemination (PCAI) is well established for pluriparous and primiparous sows, but is still limited for gilts due to the difficulty for cannula insertion (1). The PCAI allows semen dose deposition in the uterine body and consequently enables the reduction of sperm cells and the volume of the inseminating dose (1, 5). Therefore, this study aimed to compare the reproductive performance of gilts submitted to PCAI or intracervical artificial insemination (CAI) using for both techniques inseminating doses with 1.5 billion viable sperm cells in 50 mL or 2.5 billion viable sperm cells in 80 mL.

Materials and Methods

A total of 636 gilts at second estrus were randomly assigned into four groups in a 2 x 2 factorial design, considering the following factors: two artificial insemination (AI) techniques (CAI and PCAI), and two different semen doses (1.5 x 10^9 viable sperm cells/50 mL; and 2.5 x 10^9 viable sperm cells/80 mL). The PCAI was performed using a cannula specifically designed for gilts (Magaplus N®, Magapor, Zaragoza, Spain). Data were analyzed using the SAS® software 9.4. A factorial analysis has considered the effect of insemination technique, type of semen dose and their interaction using the GLIMMIX procedure. Variables including pregnancy rate (PR) and farrowing rate (FR) were analyzed using logistic regression. The averages of continuous variables such as the number of piglets born (TPB) and number of piglets born alive (TBA) were compared by Tukey-Kramer test. Only gilts in which the insertion of the cannula through cervix was possible in all inseminations were considered for analysis of reproductive performance in the PCAI group. Additionally, one gilt from the PCAI group died before pregnancy detection.

Results and Discussion

The PCAI success rate for cannula insertion through the cervix in all inseminations during estrus was 58.93% (188/319). This result confirms the difficulty for PCAI use in gilts as observed in previous studies (1, 2).

The PR, FR, TPB, and TBA were not affected ($P \geq 0.2$) by the interaction of AI technique (PCAI or CAI) and type of semen dose (1.5 or 2.5 x 10^9 sperm cells), and also, when considering the factors individually (AI technique or type of semen dose) ($P \geq 0.6$; Table 1). This is an important result since the reproductive performance was maintained even when CAI was performed with 1.5 billion sperm cells in 50 mL (the same dose used commercially in primiparous and pluriparous sows for PCAI). Moreover, just a few and older studies evaluated the reduction of the sperm cells and also the volume of the inseminating dose for CAI, which makes it difficult to compare with the current performance of the modern gilts. However, the reproductive performance observed was similar to previous studies comparing PCAI and CAI in gilts, although they used doses containing 2.5 – 3.0 billion sperm cells in 80 – 90 mL for CAI (2, 3, 4). Therefore, our results suggest the use of semen dose with 1.5 billion sperm cells in 50 mL for CAI or PCAI in gilts. However, a high quality of the semen dose and of the insemination procedure have to be provided.

Table 1. Reproductive performance of gilts submitted to post-cervical artificial insemination (PCAI) or cervical insemination (CAI) with different types of semen doses (1.5 x 10^9 sperm cells/50ml or 2.5 x 10^9 sperm cells/80ml)

	CAI		PCAI		P-*values*		
	1.5	2.5	1.5	2.5	AI[3]	D[4]	AI* D[5]
n	158	159	90	97	.	.	.
PR[1]	96.5	97.7	98.0	95.3	0.9	0.7	0.2
FR[1]	93.7	95.6	94.4	93.8	0.8	0.7	0.5
TPB[2]	14.5	14.5	14.8	14.5	0.7	0.7	0.5
TBA[2]	13.5	13.6	13.9	13.5	0.6	0.7	0.3

[1]Pregnancy rate (PR) and farrowing rate (FR), presented as percentage. [2]Total piglets born (TPB) and piglets born alive (TBA) presented as LS means. [3]AI technique (CAI or PCAI). [4]Type of semen doses (1.5 x 10^9/50 mL or 2.5 x 10^9/80 mL). [5]Interaction of AI technique and type of semen dose.

Conclusions

Success rate for cannula insertion in gilts in all inseminations was 58.93%. The reproductive performance was not affected by the use of PCAI or CAI regardless of the use of 1.5 or 2.5 billion sperm cells in the semen dose.

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Effect of oxytocin and carbetocin after expulsion of the ninth piglet on duration of farrowing and piglet performance

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Introduction

In the last decades, the increased litter size resulted in a higher number of stillborn piglets. The occurrence of stillborn piglets is associated with prolonged farrowing, mainly during the final third of farrowing (1, 2). Thus, strategies that reduce the duration of farrowing may play a role to maximize the piglet survival at farrowing. As an alternative, the use of oxytocics has been studied in different moments of the farrowing process (3, 4). The present study aimed to evaluate the effects of oxytocin or carbetocin administration after the ninth piglet born on farrowing duration and piglet performance.

Materials and Methods

A total of 316 sows were distributed in three groups, after the 9th piglet expulsion: Oxytocin (10 IU IM, n = 109), Carbetocin (0.100 mg IM, n = 113) and control (no oxytocics, n = 94). Farrowing duration (the time elapsed between the birth of the first to the last piglet), birth interval (the time elapsed between each piglet born), and the condition of each piglet were recorded. Data were analyzed with SAS (Statistical Analysis System) software version 9.4 (SAS Institute Inc., Cary, NC, USA), using GLIMMIX procedure, and the means were compared using the Tukey-Kramer test, at a significant level of 5%. The variables expressed as percentages were fitted as binomial distributed.

Results and Discussion

The farrowing duration (P=0.005) and the birth interval (P=0.028) were affected by the treatments. Lower values were observed in females from group Carbetocin, when compared to the Control group (P < 0.05), with no difference for those from Oxytocin group. The number of total piglets born did not differ among the treatments (P=0.368). The treatment did not affect the percentage of piglets born alive (P=0.161) or the occurrence of stillbirths (P=0.132). The percentage of piglets born with a ruptured umbilical cord (P=0.291) was not affected by the treatment. Control females tended to have a greater percentage of piglets born cyanotic (P=0.058), compared to those from Oxytocin and Carbetocin. However, the percentage of piglets born pale was higher in Control than in the Carbetocin group (P=0.011), with no difference for Oxytocin (P>0.268). Furthermore, in females with oxytocin and carbetocin administration, the percentage of meconium-stained piglets decreased (P <0.001), when compared to the Control group.

Table 1: Treatment effect on the farrowing duration (FrwDur, min), birth interval (BirInt, min), total piglets born (n), piglets born alive (%), and occurrence of stillborn piglets (%), rupture of the umbilical cord (RuptUmb, %), cyanotic piglet (%), pale piglet (%) and meconium staining (Mecon, %).

	Treatments		
	Control	**Oxyt[1]**	**Carbet[2]**
FrwDur	220.3±9.7[a]	202.9±9.2[ab]	182.0±9.0[b]
BirInt	15.3±0.7[a]	14.4±0.7[ab]	13.1±0.7[b]
Total born	16.0±0.3	15.5±0.3	15.4±0.3
Born alive	89.6±0.9	91.3±0.8	91.4±0.8
Stillbirth	6.6±0.7	5.8±0.6	4.9±0.6
RuptUmb	12.3±1.3	10.5±1.1	11.1±1.1
Cyanotic	3.8±0.7	2.5±0.5	2.5±0.5
Pale	3.5±0.8[a]	2.6±0.6[ab]	1.8±0.4[b]
Mecon	34.4±3.7[a]	26.6±3.2[b]	27.6±3.3[b]

[1] 10 IU oxytocin IM after the expulsion of the 9th piglet; [2] 0.100 mg carbetocin after the expulsion of the 9th piglet; [a,b] Different superscript letters within row differ significantly (P < 0.05).

Carbetocin administration reduced the total farrowing duration by 38 minutes when compared with the Control group. A longer half-life of carbetocin (85 to 100 min) (5) might explain this greater reduction in farrowing duration. Although no difference was observed in the percentage of piglets with ruptured umbilical cord, the oxytocics reduced the occurrence of pale and meconium stained piglets, which are signs of fetal stress.

Conclusions

In conclusion, administering carbetocin after the ninth piglet results in a reduction in the farrowing duration and births interval. Therefore, the use of carbetocin is an alternative to reduce fetal stress during farrowing.

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Comparative study of frozen-thawed porcine semen: homospermic vs. heterospermic doses

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Introduction

Semen cryopreservation has been successfully and efficiently used in many mammalian species; however, it is still inefficient in swine. It is well known there are high individual variations of the ejaculate among boars (1) and among sperm subpopulations (2). Individual boar sperm cryodamage could be reduced by using heterospermic doses, because some proteins and other elements present in the seminal plasma implicated in the freezability of the spermatozoa absent in one male may be present in another male, and therefore compensate for such deficiency (3). The aim of this study was to compare the effect of the heterospermic confection doses on semen parameters after thawing with the homospermic doses.

Materials and Methods

The present study was carried out using 9 ejaculates from 3 boars. Boars were housed in a climate-controlled building in a Boar Stud (CIP, Córdoba state, Argentina).
Semen was collected from 3 boars once a week by artificial vagina during three consecutive weeks. After removing the gelatinous fraction by filtration, the total volume of ejaculate was diluted in a commercial extender (MRA Antiox, KUBUS®) to a final concentration of 3 x 10^9 spermatozoa/dose. The diluted semen was split into 80 ml semen doses, which were cooled and immediately transported to the Animal Reproduction Laboratory of the Facultad of Veterinary Sciences, National University of La Plata.
In the laboratory, semen samples were cryopreserved using a modified Westendorf protocol in 6 x 10^9 sperm cells/doses. After an evaluation of homospermic samples, heterospermic samples were made at 5°C. These doses were made by pairing two homospermic doses at all possible pairs.
Homospermic and heterospermic doses were thawed in a water bath at 37°C for 1 minute and re-suspended with commercial extender (Androstar Plus, Minitube®). After thawed, semen parameters were evaluated. Total motility (MT) and progressive motility (MP) were evaluated with CASA System (MOFA®, USA). Acrosome status (PSA) was examined using fluorescent probe isothiocyanate-conjugated peanut agglutinin (FITC-PNA). Viability (V) and abnormal morphology (MORF) were evaluated using eosin-nigrosin staining technique.
Statistical analysis was performed by GLM procedure of the SAS system. Results are presented as least squares means (LSM) and standard errors (SE). Significance was set at ($P<0.05$).

Results

There were not statistical differences ($P<0.05$) between treatments. However, semen parameters (MT, MP, PSA and V) were better in heterospermic compared with homospermic doses (Table 1).

Table 1. Frozen-thaw semen parameters (LSM±SE) of homospermic and heterospermic boar semen samples.

	MT	MP	PSA	V
Homo	36.16±5.9	20.59±6.93	29.00±7.31	38.33±7.59
Hetero	42.54±2.64	27.79±3.10	33.73±3.27	45.53±3.39

Conclusions and Discussion

Using heterospermic doses could reduce cryodamage because 'Good freezability boars' gave cryoresistence to 'bad freezability boar' spermatozoa. Meanwhile no statistical differences were found among groups, standard errors values were greater in homospermic doses than in heterospermic doses, and this could explain that cryodamage could be reduced in heterospermic doses, due to less individual cryotolerance.
These results agree with those reported previously by Yeste et al (3), who demonstrated there are individual responses to cryopreservation in boar sperm cells and that boar semen cryopreservation is improved using heterospermic semen samples.

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Histopathological and cytological studies of sow endometrium

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Introduction

The inflammation of the endometrium (endometritis) is characterized by the presence of inflammatory cells and is classified based on the presence of clinical signs in clinical (CE) or subclinical (SCE). Both CE and SCE can impair reproductive performance due to reproductive failures, embryo mortality and reduction on litter size. Studies in cows and mares have shown that endometrial cytopathology is an easy, fast, safe, effective, and minimally invasive technique for diagnosing of subclinical endometritis. Therefore, the objective of the present study was to validate the use of cytopathology in sow uteruses for the diagnosis of CE or SCE using histopathology as a reference technique.

Materials and Methods

Sows (n=11) were used from a commercial farm. Genital tracts were collected in a slaughterhouse immediately after exsanguination and transported at 4°C to the College of Veterinary Sciences (National University of La Plata, Argentina). Upon arrival, samples for cytology were collected with an endocervical collector brush (Medibrush Plus®, Medical Engineering Corporation S.A.) from the uterus body and distal, middle and proximal horn areas. Samples of each brush were smeared on a slide, identified with the sow number, and stained with Romanovsky-type stains (Stain 15®, Biopur SRL). Histopathological samples were collected simultaneously one centimeter cranial from the cytological impression, by cutting a wedge section of 1cm^2 representing all the layers of the uterus. Histopathological samples were fixed for at least 24 h in 10% neutral buffered formalin, dehydrated, impregnated with, and embedded in paraffin, sectioned at 5 µm, mounted on glass slides and stained with hematoxylin and eosin (HE).

The diagnosis of endometritis was made by cytopathology and histopathology on samples collected from the uterus body (B) and distal horn (DH). The cytological evaluation was performed in duplicate at 100x. A total of 200 cells, including endometrial and inflammatory cells, were counted. Animals with a proportion of neutrophil (PN) ≥7% over the total counted cells were considered positive. A second cytological assessment was carried out considering as positive, animals that had a proportion ≥3% PN over the total number of cells (3). For histopathological evaluation, endometritis was categorized into 4 grades, based on the presence of inflammatory cells (2).

The level of concordance between both techniques was calculated by the Kappa coefficient and the McNemar statistical test was applied for statistical significance (P <0.1).

Results

Levels of concordance were better when considering a cut-off value of ≥7% PN. The highest concordance for the Kappa index (0.62) was found for DH, meanwhile for the body a moderate concordance was found. Conversely, when the average between B and DH was considered with a cut-off value of ≥7%, the Kappa index showed a moderate level of concordance (0.5) (P<0.1) (Table)

Table 1. Level of concordance between histopathology and cytopathology

	Cytopathology	Histopathology (+)	Histopathology (-)	Kappa index (95% CI)	McNemar Test
B-DH ≥7% PN	(+)	2	0	0,5	0,08
	(-)	3	17		
B-DH ≥3% PN	(+)	2	2	0,3	0,65
	(-)	3	15		
B ≥7% PN	(+)	1	0	0,42	0,16
	(-)	2	8		
B ≥3% PMN	(+)	1	1	0,23	0,56
	(-)	2	7		
DH ≥7% PMN	(+)	1	0	0,62	0,32
	(-)	1	9		
DH ≥3% PMN	(+)	1	1	0,39	1
	(-)	1	8		

Conclusions and Discussion

Endometrial cytology has previously been described for the diagnosis of SCE in bovine where a relationship with subsequent reproductive performance was found (1). Previous studies in mares also showed there is concordance with the presence of neutrophils in the cytological and histopathological examination (4). Similar results were observed in our study.

From the results obtained in this study, it can be concluded that cytological evaluation of the uterus body and distal horn can be considered a reference technique to determine the presence and percentage of PN, comparable to histopathological assessment.

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Reproductive performance of Peforelin-treated sows in a Cypriot commercial farm

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Introduction

Reproduction is a basic aspect in pig production. Optimal reproductive performance is crucial for economic success in commercial pig herds. Pharmaceuticals, such as progesterone-analogues and gonadotropins, are used in practice to control reproduction with the aim to increase the reproductive performance of gilts and sows[(1)]. Peforelin acetate is a GnRH analogue used in veterinary medicine since 2008. The positive influence of Peforelin, which induces only an FSH-increase without increasing the levels of LH, has been shown in Germany[(2)].

The specific FSH secretion by a single application of Peforelin stimulates follicular growth and helps the onset of estrus in gilts and sows. It is known that Peforelin has a positive effect to improve fertility performance especially in the warmer season[(1)]. The current study evaluated the potential of using Peforelin in Cyprus. In order to do that we recorded some important parameters which can be influenced by the use of Peforelin: farrowing rate and subsequent litter performance[(3, 4)].

Materials and Methods

Only healthy primiparous and multiparous sows, that fulfilled the inclusion criteria, were included.

Sows of the C-group received no treatment, while animals of the P-group received 24 after weaning Peforelin (Maprelin®, Veyx-Pharma, Germany) in accordance with the approved leaflet. In all sows a deep intramuscular application was performed in the left-hand side of the neck.

The following parameters were recorded: number of sows inseminated, number of farrowings, farrowing rate, piglets born alive (PBA), liveborn piglet index (= PBA/100 inseminations). The figures obtained were analyzed and compared between the C-group comprising of inseminations from October 2017 to April 2018 and the P-group with inseminations from October 2018 to April 2019. Data were collected until significance in the differences was reached.

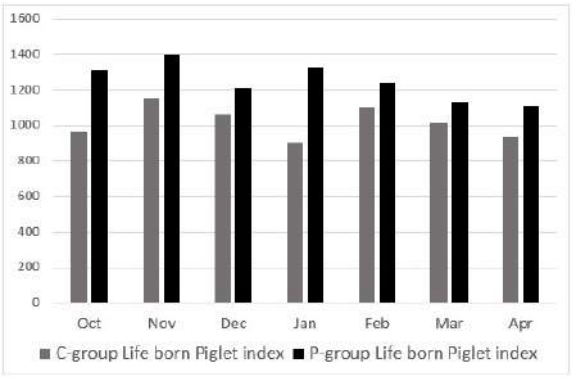
Results

A spinose of the fertility results can be found in the following table:

Table 1: Fertility results in the C-group and P-group

Group	C	P	p-value
Sows inseminated	418	276	
Sows farrowed	338	246	
Farrowing rate	80.9%	89.1%	p<0.01
PBA (total)	4219	3388	
PBA/litter	12.48	13.77	
Liveborn Piglet index*	1009	1228	

The performance per month was compared in the following graphic:



Graphic 1: Results per month in the C- and P-group

Conclusions and Discussion

Peforelin treatment had a positive influence on farrowing rate ($p<0.01$) during the study in Cyprus. As a result 217 piglets more were born alive in 100 inseminations. The number of sows analised was sufficient to obtain statistical significance.

It is recomendable to perform another study with a higher number of animals and to analise aditionally the following parameteres: piglet birth weight in different parities.

Acknowledgments

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Feeding the transition sow ad libitum: feed intake patterns and lactation performance

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Introduction

Conventional feeding strategies for transition sows limit feed intake to only 2-3 kg per day, with a gradual increase in allowance after farrowing. This is based on the fear that overfeeding may reduce feed intake in lactation, cause mammary gland oedema, and cause constipation. However, there are indications that increased feed quantities just prior to farrowing increases colostrum production (1), and that increased energy supply to the sow influences the farrowing process (2). The current study challenged conventional feeding strategies and compared ad libitum feeding prior to and during lactation to a traditional step-up feeding curve.

Materials and Methods

Multiparous sows (n = 48) and gilts (n = 33) of a commercial line (Hypor, Hendrix Genetics, Netherlands) were allocated to a traditional feeding curve or to ad libitum (ad-lib) feeding beginning a week before farrowing through to weaning (25 d), distributing parities equally across treatments. Sows and gilts on the traditional feeding curve were restrictively-fed (2.5 to 3.5 kg/d, depending on parity) before parturition, and subsequently feed allowance was gradually increased to a maximum of 8.5 kg/d. Similar to the ad lib fed sows, restrictively-fed sows were free to choose their time of feeding, but were limited in their allowance. Electronic feeders (Schauer Agrotronic, Austria) allowed portioned delivery of feed, with feeder access and intakes recorded in real time. Data were analysed using SAS (SAS® Enterprise Guide 4.3, Cary, NC, USA).

Results

Prior to farrowing, feed intake was higher in ad-lib fed sows (4.7 vs. 3.3 kg/d; $P < 0.05$) and gilts (3.5 vs. 2.6 kg/d; $P < 0.05$). During the first week of lactation, ad-lib fed sows also consumed more than traditionally fed sows (5.0 vs. 4.3 kg/d; $P < 0.03$), with intakes tending to be higher over the whole lactation period in ad lib fed sows (7.1 vs 6.6 kg/d; $P < 0.08$). In gilts, feed intake during lactation was similar for the two treatments ($P>0.10$)

No adverse effects of ad libitum feeding, such as overeating with subsequent drops in feed intake, constipation, or mammary tissue oedema were observed. Stillbirth rate was not affected by treatment (Table 1). Litters from ad-lib fed sows gained 7 kg more during lactation and their piglets were 0.5 kg/pig heavier at weaning compared to traditionally fed sows ($P < 0.05$). Average daily gains were significantly higher (247 vs 231 g/d; $P < 0.05$) over the whole lactation, and numerically higher in the first 14 d in ad-lib fed sows. In gilts there was no significant difference in litter performance.

Table 1. Lactation performance for sows fed ad libitum from entering the farrowing unit to weaning, (ad-lib) compared to sows fed to a traditional feeding curve (conv).

	PP sows		MP sows	
	Ad lib (n=17)	Conv (n=16)	Ad lib (n=23)	Conv (n=25)
Stillborn*	1.1 ± 0.3	1.2 ± 0.3	1.3 ± 0.3	1.5 ± 0.3
Piglets d1	12.9 ± 0.4	12.9 ± 0.4	14.8 ± 0.6	15.0 ± 0.6
BW, kg	1.31 ± 0.05	1.34 ± 0.05	1.38 ± 0.05	1.41 ± 0.05
# weaned	11.6 ± 0.3	12.0 ± 0.3	12.7 ± 0.3	12.8 ± 0.4
WWT, kg	6.3 ± 0.2	6.5 ± 0.2	7.6 ± 0.2[a]	7.1 ± 0.2[b]
LWWT, kg	73.3 ± 2.5	75.9 ± 2.6	97.0 ± 2.3[a]	90.2 ± 2.2[b]
Sow BW loss, kg	16.7 ± 3.0	17.5 ± 3.4	17.6 ± 3.2	19.8 ± 2.6
Sow BF loss, mm	2.6 ± 0.5	3.1 ± 0.6	2.8 ± 0.4	3.2 ± 0.4

BW: birth weight; WWT: weaning weight; LWWT: litter weaning weight; Sow BW: sow body weight; Sow BF: sow back fat; *corrected for total born; [a,b]$P < 0.05$.

Interestingly, ad-lib fed sows achieved a higher intake by spreading their intake over several meals through the day, with meal size averaging around 800-1000 g. This pattern was consistent over the whole period from before farrowing through to weaning, and consequently, ad-lib fed sows increased daily intake by increasing number of meals. In contrast, restrictively-fed sows tended to consume most of their allowance at the start of the feeding cycle in a limited number of meals. Only as lactation progressed and allowance increased, did restrictively-fed sows change their intake patterns from a few large meals (2-2.5 kg in size), to more meals averaging around 1100 g in size, similar to ad-lib sows.

Conclusions and Discussion

Clearly, feed intake capacity in transition sows is much higher than what they are generally fed, and during early lactation voluntary feed intake increases rapidly. The extra nutrient intake went to benefit milk and probably colostrum production, rather than preserve maternal reserves.

Acknowledgments

We thank the staff of Trouw Nutrition's Swine Research Centre

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Sow and litter responses to water acidification in pre-farrow and lactation

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Introduction

Providing a clean water supply throughout production promotes sow and piglet health, welfare and performance. Water acidification with a blend of free and buffered organic acids (SPH) lowers the pH of the water, limiting the growth of pathogenic bacteria.

Lactating sows consume between 20-35 L/d. This high level of water intake is important to help the animal recover the large amount of fluid lost at birth via expulsion of the placenta and birth fluids. Recovery to a normal water balance typically takes a few days. Sufficient water intake can reduce constipation issues, lower the risk of urinary tract infections and support optimal milk production during lactation. Especially in high prolific sows, water intake is of importance for sufficient milk production for the piglets. The objective of the study was to determine the impact of supplementing sows with SPH prior to farrowing and during lactation on water intake, sow performance and litter survivability and growth performance.

Materials and Methods

The study was a longitudinal randomized design with two experimental water treatments, a control (CON) and a water acidifier (SPH). Sows had unlimited access to water and the water treatments were administered from the time sows entered the farrowing room (3-5 days prior to farrow) until piglet weaning at 21 days. Sows (Hypor F1 x Hypor Magnus Duroc hybrids, n=669) were individually housed in farrowing crates and were PRRS and *Mycoplasma hyopneumoniae* negative, with no PMWS/PCV2 symptoms. All sows received the same diet, *ad libitum*. Piglets did not receive creep feed. Sow body weight and back fat were measured at farrowing and at weaning and feed intake in lactation was recorded daily. Litter characteristics at birth were recorded and weight at farrowing, after cross-foster (within treatment) and at weaning were taken. Water disappearance was measured daily to estimate sow water intake.

Sow was considered the experimental unit for growth analyses and pen was the experimental unit for water disappearance analyses. Statistical significance was declared at $p < 0.05$. Continuous data were modelled in the GLIMMIX procedure of SAS (SAS® Studio 9.4, Cary, NC, USA) with treatment and parity considered fixed effects and for litter performance, weight at cross-foster was a covariate. Batch and study were random sources of variation. Survivability data were modelled using a binomial distribution.

Results

There were no differences in total born, however, there was a tendency (p=0.09) for the ratio of born alive to total born to be higher (+1%) in SPH supplemented sows. There were no significant differences in the number of stillborn, mummies or pre-weaning mortality between treatments.

Sows supplemented with SPH drank significantly more water, 36 versus 33 L/d per sow (p=0.01). However, average daily feed intake (6.2 kg/d) was not different between treatments and there were no differences in body weight or back fat at weaning (Table 1).

There were no differences in number of piglets at cross-foster (13) or weaning (11.5). SPH tended to increase litter (p=0.07) and piglet (p=0.06) growth rates. In agreement, SPH tended to increase litter weaning weights (p=0.10) and significantly increased piglet weaning weight (=0.04) (Table 1).

Table 1. Litter and piglet growth performance

	CON	SPH	SEM	P trt
Litter Performance				
Weight cross-foster, kg	17.6	17.8	0.3	0.73
Weaning weight, kg	68.5^{y}	70.7^{x}	2.3	0.10
Average daily gain, kg/d	2.5^{y}	2.6^{x}	0.1	0.07
Piglet Performance				
Weight cross-foster, kg	1.4	1.4	0.02	0.84
Weaning weight, kg	6.0^{b}	6.2^{a}	0.1	0.04
Average daily gain, g/d	222^{y}	229^{x}	8	0.06

Note: sows in control (CON, n=339) and supplemented with acidified water (SPH, n=330). $^{a–c}$ Different superscripts within a row indicate a significant difference (p<0.05) $^{x-y}$ Different superscripts within a row indicate a trend difference (p<0.10)

Conclusions and Discussion

Sows supplemented with SPH increased water intake, without any changes in feed intake. Piglets from sows supplemented with SPH had improved growth rates and weaning weights, likely supported by improved milk production of the sow.

Acknowledgments

This experiment was performed at a commercial swine facility in Quebec, Canada. We thank the facility staff and research technician who executed the protocol.

The use of PMSG/HCG combination to support gilt development in African Swine Fever environment

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Introduction

In an environment with African Swine Fever, it is difficult to get new disease free gilts. As a result, many Chinese producers are using commercial fattening females as replacement animals instead. Compared to normal eligible gilts, fattening females are observed to have poorer reproductive performance. Commonly observed issues are delayed puberty or prolonged anestrus. In order to improve their estrus rate, effective management methods and hormones have to be applied. Follicle stimulating hormone (FSH) and luteinizing hormone (LH) act in concert to regulate the final stages of follicular development leading to ovulation. Since PMSG has biological properties similar to those of FSH, and HCG has properties similar to LH, it is physiologically appropriate to use these in combination to stimulate follicular growth in prepuberal gilts.

Key words: PMSG/HCG combinations, Gilt development, ASF

Materials and Methods

206 over 160 day's age fattening females were randomly selected from 2 farms, 101 from farm A, 105 from farm B. The average boar exposure start age of the 2 group are 170d and 165d. All the females follow the 28 – day gilt development protocol as below:

Table1. 28 – day gilt development protocol

Time	Action
Day 1 -13	Direct (and fence-line) contact with vasectomized boars
Day 14	Remix and re-pen all gilts
Day 23	All "opportunity" gilts without estrus receive PMSG/HCG-Compound
Day 28	All eligible gilts are identified Gilts without estrus are culled

All the females which were not in estrus within 23 days were treated with PMSG/HCG-Compound on day 23. The females which in estrus within 13, 23 and 28 days were recorded.

Results

Table2. Estrus rate of different time

Farm	A	B
No. females	101	105
Ave. boar exposure start age	170	165
% estrus in day 1-13	57% (58)	52% (55)
% estrus in day 1-23	68% (69)	60% (63)
% estrus in day 1-28	87% (88)	85% (89)
No. females treated with PMSG/HCG-Compound	32	42
% estrus induced by PMSG/HCG-Compound	59% (19)	62% (26)

Conclusions

The result shows that proper gilt development protocol and the application of PMSG/HCG combinations can assist producers with getting good gilt heat rates, even in the situation of using fattening females as replacements.

28-day gilt development protocol with PMSG/HCG combinations could be an efficient solution for estrus induction in prepubertal gilts especially in some emergency situation such as African Swine Fever.

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Comparing PG600® and Gestavet® on estrus induction in gilts selected from fattening pigs

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Introduction

Follicle stimulating hormone (FSH) and luteinizing hormone (LH) act in concert to regulate the final stages of follicular development leading to ovulation. Since PMSG has biological properties similar to those of FSH, and HCG has properties similar to LH, products containing both ingredients can stimulate follicular growth and ovulation in prepuberal gilts. Many producers are using fattening gilts as replacement animals as it is difficult to get new disease free gilts during African Swine Fever outbreak. Compared to gilts from specialized genetic lineage, fattening females are observed to have poorer reproductive performance. In order to improve their estrus rate, effective management methods and hormones have to be applied. The effect of PG600 and Gestavet were compared.

Key words: PMSG/HCG combinations, Gilt development, ASF

Materials and Methods

2121 over 180 day's age fattening females were randomly selected. All the females follow the 28 – day gilt development protocol as below:

Table 1. 28 – day gilt development protocol.

Time	Action
Day -13	Direct (and fence-line) contact with vasectomized boars
Day 14	Remix and re-pen all gilts
Day 23	All "opportunity" gilts without estrus receive PMSG/HCG-Compound
Day 28	All eligible gilts are identified Gilts without estrus are culled

On the 23rd day, 488 gilts that had not shown estrus were separated into two groups, one group (295 gilts) was treated with PG600, while the other group (193 gilts) was treated with Gestavet. Gilts which in estrus within 13, 23 and 28 days were recorded.

Results

After boar exposure, 1633 of the 2121 gilts presented estrus within 23 days. Result of the rest 488 gilts which were treated with PG600/Gestavet as shown in the table below:

Table 2. Estrus rate after the treatment with two products.

Product	PG600	Gestavet
No. Gilts be treated	295	193
% Estrus after treat	70.17% (207)	64.77% (125)

P = 0.211, Chi-Square analysis

Conclusions

The result shows PMSG/HCG combinations are very efficient in estrus induction in prepubertal gilts selected from fattening females and may be a good choice for producers who are using fattening gilts as replacement animals during the African Swine Fever period. The findings suggest differences in zootechnical performance of different compounds available to producers, however the study would have been improved with larger sample sizes. Unfortunately due to the nature of the production system, increasing sample size in each group was not possible. PG600 was numerically superior in terms of ability to induce gilt heat.

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The relationship between serum anti-Müllerian hormone concentrations and the ameliorative effects of exogenous gonadotropins on gilts with delayed puberty in a farm in Thailand

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Introduction

The replacement gilts, in Thailand, reach puberty at approximately 200 days of age (1). The gilts attaining puberty late can possess a poor number of lifetime reproductive performances (2) and are removed from herds earlier than they should be (3). The previous study reports that gonadotropins containing 400 IU eCG plus 200 IU hCG could significantly improve the number of gilts with estrus, onset of estrus expression, the number of dominant follicles, and farrowing rate in the gilts with delayed puberty problem (4). Anti-Müllerian hormone (AMH) was first report in the rabbit embryos and play the important role of the breakdown Müllerian ducts and development of male characteristics. In females, AMH is now also associated with ovarian antral follicle reserves in cattle (6). The serum AMH and E2 levels were inform that associated with the selection of gilts for the breeding herd. It is interesting whether the AMH levels in gilts with delayed puberty are different from those of normal gilts and are related to the effects of gonadotropin stimulation. The aim of this study is to find the relationship between serum anti-Müllerian hormone concentrations and the ameliorative effects of exogenous gonadotropins on gilts with delayed puberty.

Materials and Methods

Delayed prepubertal (>200 days of age) cross-bred (LY) gilts (n=32) were used. At about 140 kg bodyweight, a blood sample was obtained for progesterone assay to confirm prepubertal status, and AMH concentrations, and then the gilts were injected with 400 IU eCG plus 200 IU hCG (PG600®). From 2 days after injection, estrus detection was performed by back pressure test in association with fenceline boar contact. All gilts were check prepubertal status by serum progesterone level at 10 day. Serum AMH concentration was compared between pubertal gilts and anestral gilts using student t-test. The significant level was limited at $P<0.05$.

Results

Two gilts were excluded from this study caused from they are puberty at the onset of experiment. In total, 27 (90%) gilts were puberty after 10 days of PG600 injection, with 3 (10%) gilts remaining anestrus at 10 days. The serum AMH level of the pubertal group was higher than the AMH level of the anestral group (36.1±1.8 vs 25.4±1.9 ng/mL; $p\leq0.001$).

Discussion and Conclusions

Previous study showed that 92% of gilts with delayed puberty were puberty after injection of gonadotropin (4) which supports our data showing 90% of gilts are puberty within 10 days. The higher AMH levels observed in pigs could be attributed to this species being polyovular and having a relatively large follicular reserve and antral follicle count and ability to be a puberty (6) agreed with observing in this study. The serum AMH level of the pubertal group is significantly higher than the anestral group. From the results in this study, AMH may play an important role in the onset of puberty in gilt.

Acknowledgments

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The relationship between serum anti-Müllerian hormone concentrations and the estrus response of gilts to exogenous gonadotropins

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Introduction

Inducing estrus in prepubertal gilts with exogenous gonadotropins most commonly uses a combination of 400 IU equine chorionic gonadotrophin (eCG) and 200 IU of human chorionic gonadotrophin (hCG). While effective, injection of gonadotropin does not result in a 100% estrus response. Frequently, only 70% of prepubertal gilts display estrus 7 days (1). In general, gonadotrophin injections into gilts of 150-160 days is appropriate to stimulate estrus and subsequent cyclicity (2,3). Early culling of gilts is most likely due to the absence of estrus. Anti-Müllerian hormone (AMH) was first found in the rabbit embryos and caused the breakdown of Müllerian ducts and development of male characteristics. In females, AMH is now also associated with ovarian antral follicle reserves in cattle (4). The relationship between circulating AMH and follicle populations in pigs is yet to be adequately described. There is evidence that indicates AMH may have a unique role in pigs. AMH continues to be expressed at similar levels to the antral stages and intensifies in preovulatory follicles. Based on studies that have previously been reported, AMH levels in the blood of prepubertal females have a strong influence on the functioning of the FSH and LH hormones to stimulate the formation of follicles and ovulation. Therefore, we hypothesised that circulating AMH levels in prepubertal gilts will affect their estrus response to exogenous gonadotropins.

Materials and Methods

Prepubertal cross-bred (LY) gilts (n=152) were used. At about 165 days of age and 100 kg bodyweight, a blood sample was obtained for progesterone assay to confirm prepubertal status, and AMH concentrations, and then the gilts were injected with 400 IU eCG plus 200 IU hCG (PG600®). From 2 days after injection, estrus detection was performed by back pressure test in association with fenceline boar contact. Gilts expressing estrus within 7 days after injection were examined by ultrasonography to determine numbers of preovulatory follicles. Serum AMH levels in the first 25 gilts to show estrus (fastest), and the last 28 gilts (slowest), were compared and the number of pre-ovulated follicle,s using student t-test. The significant level was limited at $P<0.05$.

Results

In total, 112 gilts showed estrus within 168 h of PG600 injection (Fig 1), with 10 gilts remaining anestrus at 10 days. The serum AMH level of the fastest group was higher than the AMH level of the slowest group (52.6±9.2 vs 39.8±6.1 ng/mL; p=0.04). The fastest group also had more preovulatory follicles than the slowest group (13.3±1.3 vs 11.4±1.2; $P\leq0.0001$)

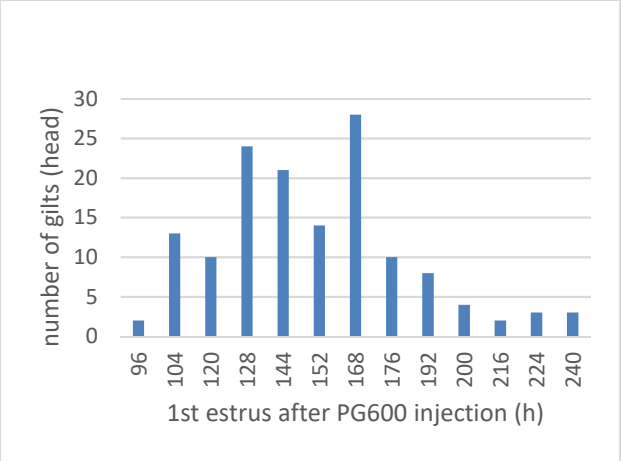

Figure 1. Distribution of interval to estrus after PG600 injection

Discussion and Conclusions

In many species (5,6), circulating gonadotropin levels are correlated with serum AMH concentrations and AMH was reported to upregulate FSH secretion and pituitary FSHb transcripts to enhance folliculogenesis (5), which supports our data showing a relation between the number of preovulatory follicles and serum AMH concentrations. The present data suggest that serum AMH levels has the potential to be used as a marker for gilt selection before induction puberty using PG600.

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Reproductive tools to reduce nonproductive days (NPD) and improve performance, inducing estrus in gilts/sows with reproductive failure

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Introduction

Efficiency is a critical component of livestock production. Sows who fail to show estrus lead to increased NPD's or need to be culled. As a result, the farrowing batches are not composed as planned, leading to financial losses. Gonadotropin hormone treatments can help to induce estrus. Objectives of this study were: 1. investigate the effect of this treatment in problematic sows, 2. to get insights into different gonadotropin treatments. (1)

Materials and Methods

This experiment was conducted in two Spanish farms (1250 and 2200 sows). We included gilts/sows that had no heat, weaned sows >7 days without estrus, and non-pregnant sows (identified by ultrasound). Total of 104 animals were randomly assigned to two groups, and treated as follows: Group A, PG600® and Group B generic gonadotropins. Progesterone levels were determined for all sows (Ovu-check®, Biovet), (2). Treatment results were statistically analyzed using Chi-Square and Unilateral Fisher tests

Results

Estrus of weaned sows with no signs 7 days after was at Farm1 75% (n=12), and 25% (n=8) for A and B groups respectively. And for Farm 2, 22% (n=18) 0% (n=5), for A and B groups respectively p=0.04

Non pregnant sows were for Farm 1, Group A 82% (n=11) (it was decided used only treatment A in these group of sows)

Looking to progesterone results: (considering as negative < 2.5 ng/ml), and % of estrus post-treatment we found Farm 1, negative 30% (100% estrus) and 62% (20% estrus), for groups A and B respectively. For Farm 2 negative 50% (25% estrus) and A, 0% estrus B). Gilts B negative 70% (29% estrus), A negative 80% (63% estrus)

Discussion and Conclusions

Use of gonadotropins and determination of progesterone levels can be very useful tools to control the sow's reproductive status and gonadotropins can induce heat in delayed animals. This reduces non-productive days and improves productivity and farm organization. In his study, PG600® has demonstrated to be more effective in inducing estrus with statistical differences compared to a generic product.

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Effects of uterotonics on farrowing performance: a meta-analysis

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Introduction

Oxytocin is the most used, often misused, uterotonic agent to control the farrowing process (1). More recently, carbetocin, a long acting analogue of oxytocin, have been introduced. Uterotonics are commonly used to reduce farrowing duration since prolonged farrowings are linked to greater stillbirth rates (2) However, albeit conflicting, evidence shows that treatment with uterotonic may increase the risk of dystocia and in the incidence of perinatal mortality. Therefore, a meta-analysis was conducted to evaluate the effects of oxytocin and carbetocin on farrowing performance.

Material and Methods

Two researchers individually performed an electronic-based search for studies using the PubMed, Web of Science, Science Direct, Scopus, and SciELO databases, during April 2020. A total of 17 papers were selected based on the criteria: evaluation of oxytocin or carbetocin, farrowing traits, peer reviewed articles written in English and published between 1990 and 2020The variables tested were extracted from both "Material and Methods" and "Results" sections of each article. The primary outcomes were total born, born alive, stillborn, farrowing duration, birth interval and farrowing assistance. All statistical analyses were performed using the software Minitab (Minitab for Windows, v. 18, USA). The meta-analyses followed three sequential analyses: graphical assessment, testing for correlations, and variance analysis. The code of study effect was considered in all analytical models as a random effect.

Results

Table 1 summarizes the outcomes of the present study. The use of oxytoxin reduced the farrowing duration by 19% and the birth interval by 21% compared to control, while carbetocin reduced the same aspects by 27 and 23%, respectively ($P < 0.001$). Nonetheless, oxytocin increased by 28% ($P < 0.05$) the stillbirth rate compared to control. The stillbirth rate was not affected by carbetocin. In addition, oxytocin and carbetocin increased the farrowing assistance by 81 and 56%, respectively, compared to control ($P < 0.05$).

Table 1. Effects of oxytocin and carbetocin on farrowing performance

	Treatment		
Responses	Control	Oxytocin	Carbetocin
TB (n)	11.77	11.78	11.45
BA (n)	10.98	10.918	10.65
SB (%)	6.26[a]	8.03[b]	6.29[a]
FD (min)	235.99[a]	190.35[b]	172.48[b]
BI (min)	20.62[a]	16.23[b]	15.97[b]
FA (%)	13.58[a]	24.53[b]	21.14[b]

TB: total born per litter; BA: born alive per litter; SB: stillborn per litter; FD: farrowing Duration; BI: birth interval; FA: farrowing assistance
Within a row, means followed by different letters differ ($P < 0.05$) according to Tukey's test.

Discussion and Conclusion

Although oxytocin significantly reduced farrowing duration and birth intervals, there was an increase in the percentage of stillborn piglets. This effect probably is due to the increased number and severity of uterine contractions, which may enhance the fetal asphyxia (2). Carbetocin treated sows, however, had decreased farrowing duration and birth interval without effects on stillbirth rate. Furthermore, both treatments increased the necessity of farrowing assistance. It is noteworthy that farrowing assistance through vaginal palpation is a risk factor for puerperal diseases and can contribute to increase farrowing duration (3). Despite the positive effects of uterotonics on farrowing kinetics, its utilization may have detrimental effects on productivity and animal welfare. Therefore, its indiscriminately utilization must be avoided. Finally, more studies are necessary to evaluate the effects of different dosages and moments of application throughout the farrowing process.

Acknowledgments

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Benchmarking of the quality of seminal doses from different European boar studs

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Introduction

External quality control programs carried out by central laboratories have been long established in human andrology with the aim of enhancing the accuracy and reproducibility of semen assessment (1). In the commercial boar studs it is common to perform the analysis of motility, sperm concentration and abnormal forms. An external quality control is vital to make sure that the doses delivered to costumers are in optimal conditions. With this purpose, Magapor launched the Seminal Quality Monitoring and Control Plan. Directed towards artificial insemination centers from different European countries (mostly Spanish), the monitoring plan is based on the periodic analysis of a constant percentage of the number of boars present at the boar stud (15%).

Materials and Methods

The descriptive study is based on the semen doses analyzed from more than 40 artificial insemination centers during a period of 7 years (2013-2019). Parameters evaluated within this monitoring plan are similar to those evaluated in other countries in which the pig sector is also important (2). Inside this plan the following parameters are analyzed (average, distribution and percentage of doses within optimal ranges): reception temperature, pH, osmolality, conductivity, weight, sperm concentration and coefficient of variation, motility, agglutination, abnormal forms, acrosomes, membrane integrity and function tests (sHOST) (3), microbiology (total mesophilic aerobes and molds and yeasts).

Results

Parameters that have evolved a more relevant improvement during the last 7 years: minor decrease in concentration in spz x 10^6 / ml, with a reduction of 7 points in the coefficient of variation. Increase of 6 points of total motility (81% to 88%) and decrease from 25% of abnormalities to 17.5%. Increase of the membrane and acrosome integrity from 69% to 78.5% and 90% to 94.53% respectively. Regarding to the percentage of doses within optimal ranges, the most significant progress has been related to the morphology, being in 2012 only the 67.93% of the doses with a percentage of abnormalities under the 30% and in 2019 the 92.31%. The biggest percentage of doses with any of the parameters out of the ranges are summer months, increasing up to the 20%.

Conclusions and Discussion

The seminal doses produced in the European boar studs have substantially improved during the last years the values of the parameters evaluated. It is considered that this improvement is related to artificial insemination centers technification measures carried out for the last years (4): use of CASA systems, introduction of Standard Operation Procedures (SOPs), staff training and high performance extenders … helping all of them to avoid many factors that could cause changes potentially reducing the fertility potential (5) and improving the quality of the doses manufactured. Although, as the results show there are still opportunities for quality improvement during the summer period.

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Could altrenogest supplementation from day 6 to 12 of pregnancy modulate uterine glandular development and growth promoters secretion around implantation?

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Introduction

Progesterone regulates the secretory activity of endometrial glands during the peri-implantation period stimulating the uterine secretion of numerous molecules, which sustain conceptus development (1). Among these, growth factors, namely IGF-1 and VEGF, are highly expressed around implantation, exerting influence on conceptus growth/development as well as on endometrial architecture (2). Indeed, IGF-I concentration is positively correlated to fetal weight and is lower in IUGR pig conceptuses (3) Thus, this experiment was designed to test the hypothesis that progestagen supplementation from d 6-12 of pregnancy improves endometrial glandular development and increases endometrial gene expression of IGF-1 and VEGF around implantation.

Materials and Methods

A total of 12 females, (6 sows and 6 gilts) were used. Treatments were arranged in factorials (2 x 2), with two categories (sow or gilt), and two treatments (non-supplemented or altrenogest-treated). On d 6 of pregnancy females were allotted at random to one of the following groups: non-supplemented (NS; n = 3 sows and 3 gilts); supplemented with 20 mg of altrenogest from d 6-12 of pregnancy (ALT; n = 3 sows and 3 gilts). Females were slaughtered on d 13 of pregnancy and samples of endometrium were collected for quantification VEGF and IGF-1 by real-time PCR. Samples of uterine tissue were obtained to measure glandular density (number of glands divided by the area of endometrium) and mean glandular area (the average area of 50 glands per photomicrograph) by histology. Data were analyzed using the PROC GLM function (SAS Inst., Cary, NC USA, 2002). The LSD test was used to evaluate the treatment and/or category effect.

Results

The results of endometrial IGF-1 gene expression are presented on figure 1. A tendency for increased (P = 0.070) VEGF gene expression was observed in the ALT group compared to NS-group, irrespective of category. The results of endometrial glandular epithelium are presented on table 1.

Discussion and conclusion

It has been demonstrated that sows supplemented with progestagen from d 6-12 of pregnancy had larger and heavier embryos compared to non-supplemented sows (4). In this study, altrenogest treatment from d 6-12 of pregnancy resulted in the hyperplasia of endometrial glands; furthermore, a tendency for increased VEGF expression and increased expression of IGF-1 were noted, remarkably for sows, which presented a 56-fold increase in the expression of IGF-I compared to NS-sows. Considering that maternal secretions, including IGF-1, during early pregnancy determine the final size of elongated embryos and consequently further embryonic development (1, 3), these results suggest that the hyperplasia of uterine glands and increased expression of endometrial IGF-1 induced by altrenogest supplementation from d 6-12 of pregnancy could be used as a tool to improve pigs' embryo development.

Table 1. Effects of altrenogest supplementation on endometrial glandular epithelium in sows and gilts.

Variable	Category	Groups	
		NS	ALT
GD	Sow	112	102
	Gilt	116	120
	Mean	113 ± 6.4	111 ± 8.2
MGA	Sow	1431	1916
	Gilt	877	1072
	Mean	1168 ± 106.7[b]	1471 ± 122.1[a]

[ab] Different superscripts indicate P < 0.05.
GD – Glandular Density; MGA – Median Glandular Area

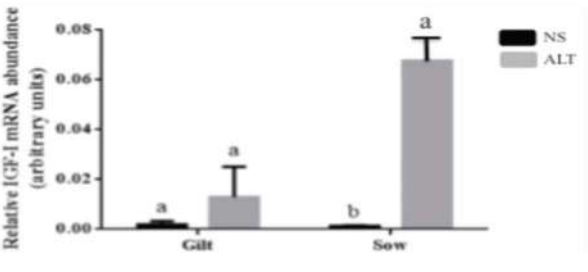

Figure 1. Expression of IGF-I and on endometrium of sows and gilts supplemented with altrenogest. Different superscripts indicate P < 0.05.

Acknowledgments

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Effect of hydroxy-selenomethionine on boar's seminal doses production and quality

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Introduction

Selenium (Se) supplementation in the diet has proved to be an essential factor for sperm cell development and maturation, besides composing essential antioxidants able to influence semen quality and male fertility (1). Selenium uptake, utilization, and storage by the organism are dependent on several factors, such as the form in which it is supplemented (i.e. inorganic selenium; organic selenium compound). The greater benefit of hydroxy-selenomethionine (OH-SeMet), a pure synthesized form of organic selenium, compared to conventional inorganic ones has been demonstrated in different species (2,3,4). Therefore, the present study aimed to evaluate the effects of dietary supplementation of OH-SeMet on boar's seminal doses production and its quality.

Materials and Methods

Forty-six boars were divided into the following treatments: SS3 (0.3 mg kg^{-1} sodium selenite (SS); n=23) and OH-SeMet3 (0.3 mg kg^{-1} OH-SeMet -Selisseo®, Adisseo; n=23). The animals received daily the different experimental diets for 95 days. To ensure animal intake of experimental diets, those diets were first provided to the animals in a volume of 0.3 kg, before a basal diet without the addition of any source of selenium was provided to all animals to reach 2.7 kg boar day^{-1}. Boar ejaculates were analyzed fortnightly for volume, sperm concentration and motility using Computer-Assisted Sperm Analysis, CASA (AndroVision®, Minitüb GmbH, Tiefenbach, Germany). Data were analyzed using the MIXED procedure (SAS, version 9.3) according to a blocked design containing treatments as main factors. Each animal was considered as one experimental unit. Treatments were evaluated using PDIFF test.

Results and Discussion

The semen volume and total motility did not differ between the treatments (P>0.05). The sperm concentration, total sperm per ejaculate and number of seminal doses produced from OH-SeMet3-fed boars was statistically superior (P<0.05) as compared to SS3 (Table 1).

The data demonstrated that boars supplemented OH-SeMet have their sperm production increased when compared to SS, probably due to its higher capacity to be stored in animal tissues (2,3,4), increasing its presence in the male reproductive tract and improving sperm cell development and maturation in addition to selenoproteins synthesis to a better antioxidant status of the boars. The higher number of total sperm per ejaculate, as well as the number of seminal doses produced from it, are in accordance with another study that compared the use of organic selenium compound against sodium selenite (5).

Table 1. Values (mean) of semen characteristics in the boars supplemented with different selenium sources

	Treatments		SEM	P-value
	SS3	OH-SeMet3		
VOL	230.03	202.62	4.57	0.4385
SC	264.24^{b}	346.94^{a}	7.30	0.0368
TSE	55.99^{b}	63.36^{a}	1.14	0.0468
SD	18.66^{b}	21.12^{a}	0.38	0.0474
TM	87.42	88.54	0.26	0.0821

VOL, volume (mL); SC, sperm concentration ($\times 10^6$ sperm/mL); TSE, total sperm per ejaculate ($\times 10^9$ sperm); SD, seminal doses produced per ejaculate (3×10^9); TM, total motility (%). ^{a,b}Different letters in the same row indicate a significant difference between treatments (P < 0.05).

Conclusions

Supplementing boar diets with OH-SeMet increases sperm concentration in the ejaculate and allows more seminal doses to be produced.

Acknowledgments

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Use of natural extracts like swine extender for their encapsulation with silver nanoparticles as an antimicrobial agent in alginate beads

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Introduction

The boar semen encapsulation in alginate beads is one of the main innovations in Artificial Insemination (A.I.) techniques. In this methodology usually used of the seminal doses is with commercial- swine extender (1). However, a low sperm viability it has been seen once that the spermatozoa were released of the alginate beads, because of that we propose the use of natural extracts like swine extender (2,3). On the other hand, due the bacterial load in the seminal doses and the resistance of them for the excessive use of the antibiotics commonly uses, for those reasons we propose the incorporation of Silver Nanoparticles (AgNPs) like antimicrobial agent against of two principal strains: *Pseudomonas spp.* and *Staphilococcus aureus,* which are related with reproductive problems in the pigs (4).

Materials and Methods

The seminal doses were collected from a Yorshire boar. The natural extracts were synthetized with two different dehydrate leaves of plants: *Cymbopongo citratus* (Cc) e *Hipericum perforatum* (Hp). The synthesis of AgNPs were made with green chemistry using like reducing agents the natural extracts and 1 mM of Silver nitrate ($AgNO_3$) in 25 ml of distilled water in two different solutions. According to a previously reported method of Torre, M.L., Barium chloride (0.07M), Hydroxypropyl-metylcellulose (HPMC) (0.03gr) and the natural extracts with the seminal doses were dropped into a Sodium alginate (0.5%) solution with AgNPs to obtain alginate beads with liquid matrix. The evaluation of sperm motility was determined by Optical microscopy and by a Seminal Quality System (SQS). The AgNPs solution was evaluated by UV-Vis spectroscopy, Scanning and Transmission Electron Microscopy (SEM and TEM) for the exact size of the NPs and their antimicrobial effect in two different strains: *Pseudomonas spp.* and *Staphilococcus* in TBS culture media. The size and the morphology of the beads were analyzed by SEM.

Results

The viability of the seminal doses was analyzed previous of the encapsulation while obtained 80% of viability. The samples of AgNPs were analyzed by UV-Vis spectroscopy gave an absorbance peak between 440 nm for *Cc* and 460 nm for *Hp* according to the literature for silver colloid solutions, the presence of NPs in the surface of the alginate beads by SEM with the technique of backscattered electrons (fig.1b) and their size lower than 50 nm by TEM (fig.1c). The antimicrobial effect of the AgNPs was evaluated by well diffusion method obtaining inhibition halos between 0.34 and 0.64 cm after their incubation. The interaction and the seminal viability with both natural extracts were evaluated for two consecutive days, we observed their viability at 5% after 48 hours. Finally, we obtained a good percentage of motility for the spermatozoa after being released from the alginate beads with liquid matrix (fig.1a).

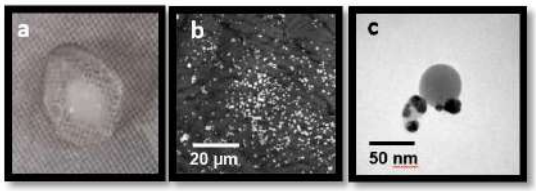

Figure 1. a) alginate bead with liquid matrix with boar semen, b) AgNPs in the Surface of the alginate bead with backscattered electrons by SEM and c) Size of AgNPs by TEM less than 50 nm.

Conclusions and Discussion

The encapsulation was achieved in alginate beads with a liquid matrix of Silver Nanoparticles, that have antimicrobial properties which are released into the sow reproductive tract acting against specific strains reducing reproductive problems, and semen in natural extracts maintaining an appropriate sperm viability for their use due to the antioxidant properties of the plants chosen to be released at specific times for 3 consecutive days due to temperature destabilization of the alginate membrane.

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Detection of intrauterine produced antibodies and PCR testing in stillborn piglets

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Introduction

Detection of antibodies in still born piglets can prove intrauterine infection. The objective of this study is to analyze whether the immunocrit assay can detect antibodies in stillborn piglets.

Materials and methods

Over a half year period, piglets submitted for routine diagnosis for stillbirth (SB) (farms with >8% SB) were included in this study. In total, 17 farms submitted fresh stillborn piglets. Stillbirth was confirmed by necropsy (non-respired lungs, non-milk containing stomach). From the heart serum blood was obtained and serum was separated and freezer stored (-80 °C) upon analysis. Serum was analyzed for antibodies, using the golden standard Radial Immuno Diffusion (RID; Triple J Farms swine IgG). The samples were alternatively tested with the immunocrit assay (1), however with an increase of centrifugation time of 10 minutes. Per farm, lung, liver and spleen were collected and pooled per 5 for PCR testing of PPV, PRRS, PCV2 and PCV3.

Results

In total, 157 still born piglets obtained from 15 farms were analyzed with both IC as RID. From these 157 samples, 13 samples, obtained from 3 farms, were positive for swine IgG using the RID assay (8.3%; 0.4-13.5 mg/ml; farm 1, 3 & 11). When using the immunocrit assay, 31 samples were positive (mean IC ratio 0.03; 0.01-0.08). When a cut off value of IC 0.02 was used (<IC 2.0 neg; >=IC 2.0 pos), this resulted in a sensitivity of 92.3% and a specificity of 94.4% (Table 1).

Table 1 Cross comparison of RID (Golden standard) and the immunocrit assay for the detection of antibodies in serum of still born piglets. A cut off value of IC-ratio 0.02 was used. This resulted in a sensitivity of 92,3% and a specificity of 94,5%.

		Radial Immuno Diffusion		
		Pos	Neg	total
IC	Pos	12	8	20
	Pen	1	136	127
	total	13	144	157

Pcr testing: in 2 of the 15 farms SB piglets were positive for PRRS**,** 1/15 farms were positive for PPV, 9/12 farms were positive for PCV2 (

Table *2*).

Table 2 PCR testing results. Farm 1, 3 and 11 were also positive for swine IgG antibodies

Farm	PRRS	PPV	PCV2	PCV3
1	Neg	Neg	**Pos**	Neg
2	Neg	Neg	**Pos**	Neg
3	Neg	Neg	Neg	**Pos**
4	Neg	**Pos**	**Pos**	Neg
5	Neg	Neg	Neg	Neg
6	Neg	Neg	**Pos**	Neg
7	Neg	Neg	Neg	Neg
8	Neg	Neg	Neg	Neg
9	Neg	Neg	Neg	Neg
10	Neg	Neg	Neg	Neg
11	Neg	Neg	**Pos**	Neg
12	**Pos**	Neg	Neg	**Pos**
13	Neg	Neg	**Pos**	Neg
14	**Pos**	Neg	**Pos**	Neg
15	Neg	Neg	Neg	Neg
Total Pos	2	1	7	2

Elisa testing for the present of antibodies against PCV2 and PRRS were negative. There was not enough serum for PPV antibody testing.

Discussion/Conclusion

Prevalence of infections in stillborn piglets is hardly reported. In total at 9/15 farms, positive PCR tests proved the presence of intrauterine infections with either PRRS, PPV, PCV2 and/or PCV3. The relevance of the presence of PCV3 is, however, still unclear (2). The immunocrit assay proved to be a useful alternative for predicting the presence of intrauterine produced antibodies, which could be an indication of intrauterine infections related to SB. The test is fast and cheap and can be used before more expensive ELISA testing for specific infections is performed. With negative AB and PCR results, noninfectious causes of SB are more likely (6/15 cases).

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Effect of semen collection frequency and breed on semen characteristics of artificial insemination boars in a commercial system in Colombia*

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Introduction

Pig genetic transfer centres in Colombia provides genetic material, usually from maternal and terminal type lines of high genetic merit, that pursue establish desired characteristics in an emerging market. However, according to management and racial characteristics, each line (Crossbreed) can differ in characteristics as sperm quality, particularly in individuals with maternal performance. In our country, reports of sperm quality information of pigs are unusual. Therefore, the objective of this study is to know the effect on ejaculate volume, sperm concentration, sperm abnormalities (CASA) and dose number per ejaculate, of semen collection frequency (SCF) and line characteristics: maternal: Landrace (LD) and Large White (LW), and terminal: Pietrain x Duroc x Landrace (PDL) Pietrain x Duroc (PD).

Materials and methods

A retrospective study of 390 boars were done. After random sampling, 120 boars (3000 ejaculates), were distributed in 4 lines analysed: (LW) (LD), 447 (PD) y and (PDL) (30 boars/breed) through 25 semen collections per boar. Each ejaculate were analysed for seminal volume (SV) (ejaculate volume/ml), sperm concentration (SC) ($1x10^6$/ml), sperm motility: masal (MM) (%) and progressive (PM) (%), sperm abnormalities (%) (SA) (% of abnormal spermatozoids), and artificial insemination dose number / ejaculate (SD). A repeated measurements mixed model were run for intrasubject variation (25 semen collections) (SCF) and for intersubject variation, a univariate mean analysis (boars/breed) with One-Way Anova for SV, SC, MM, MP (Media±SEM), and SA (Median±IQR) (SPSS-23®)

Results

No variables present sphericity ($p \leq 0.01$) accepting Greenhouse-Geisser test for SCF effect, being significant to intrasubject variation for MM, PM and SD (p0.05), and equal for SV and SD ($p \geq 0.05$). Also, lines interact significantly with SCF, influencing SV, SC, MM, PM and SD (p0.01). SA were influenced by line effect (p0.05) and not influenced by SCF effect ($p \geq 0.05$). In terms of intersubject variation, when SV were analysed (PD) present higher SV (266.5±10.1) compared to the other lines (209.6±7.5 - 214.1±9.6 and 225.1±8.9) LD, PDL and LW respectively ($p \leq 0.05$). In terms of SC and MM no differences were found with ranges of 16.7±0.5 - 18.05±0.5 and 92.8±0.3 - 93.7±0.1, respectively ($p \geq 0.05$). On PM, PD line present a higher individual motility (87.1±0.4) that other ones (86.3±0.3 - 85.0±0.5 and 85.3±0.6) LD, PDL and LW respectively ($p \leq 0.05$), that also were reflected on a higher SD (26.0±0.9) when compared with LD, PDL and LW (20.0±0.9 - 19.0±0.8 - 20.0±0.6) ($p \leq 0.05$). SA of PD line were lower (2.53±1.17), than LD, PDL and LW respectively (3.9±1.9 - 3.9±1.8 and 4.6±3.9) (Median±IQR) ($p \leq 0.05$).

Discussion and Conclusions

Available information of sperm quality (SQ) variability of boars for commercial systems in Colombia is rare, limiting criteria to understand SQ variation in the tropics. MM and SC in all genotypes were similar, exceeding 90% of MM and 16-18x10^6 spz/ml respectively, considered higher in comparison with other systems (75% MM) (3, 4). PDL as a trihybrid has muscle depth and fat reduction traits, than can reduce SV and SC. PDL considered terminal, present lower SV than PD, suggesting that sperm quality traits segregate independent of heritability (1, 4), and breed effect influence semen quality parameters (1). In conclusion, genotype is a main cause of variation of SQ, and PD as terminal line present better SQ, when compared to maternal lines, these differences may be result of selection processes for desirable productive traits, but not for secondary traits as SQ, and for maternal lines in especial LW stress influence on results (2).

Acknowledgments

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Ovarian morphometrical evaluation to assess reproductive activity suppression in heavy weight finishing gilts immunized against gonadotropin-releasing hormone

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Introduction

The rearing of heavy market weight gilts (over 120 kg) is challenging, as growth and feed intake decrease during the periods of estrus, which causes great economic losses to producers (1). Immunocastration emerged as an alternative method to surgical castration, as it inhibits signs of estrus, improving weight gain (1).

Even though there is evidence of ovarian weight commitment in immunized gilts (2), there is lack of information on ovarian histomorphometrical alterations. Thus, the objective of this study was to evaluate the impact of immunocastration on ovarian morphology in heavy-weight finishing gilts using histomorphometrical approaches.

Materials and Methods

Eighteen prepubertal gilts were randomly selected and allocated to the following experimental groups: IM (n = 6; gilts which received two injections of 2 mL of Vivax®: at 15 and 19 weeks of age) and CT (n = 12, females which received two saline injections, following the same protocol). Animals were slaughter at 25 weeks of age, the ovaries were collected and processed for histomorphometrical evaluation.

Ovarian follicles were divided in different classes, according to the morphological characteristics established by Ross & Pawlina (3). The areas of the histological sections were measured using the ImageJ® software, and their average was used to calculate the number of follicles per area. Secondary and mature follicles' diameters, as well as the diameter of their antrum, were measured using a graduated ruler. These values gave rise to the following classification of mature follicles: F1 (≤ 1 mm), F2 (1.1 – 2.0 mm), F3 (2.1 – 3.0 mm) and F4 (≥ 3.1 mm). The area of the granulosa layer was also determined, corresponding to the difference between the follicle area and the antrum area, which were calculated using the circumference area's formula (A = π r^2). Treatment effects were established by ANOVA and comparisons between means done by the Student T test

Results

A higher number of primordial and primary follicles per area and final atretic follicles were observed in IM animals ($P < 0.05$). However, immunocastration did not affect the number of secondary and mature follicles. When mature follicles were classified by diameter, even though F1 and F2 were not affected by vaccination against GnRH, F3 and F4 follicles were absent in the ovaries of IM gilts ($P < 0.05$). Regarding granulosa layer area, it was similar in secondary follicles, but larger areas were observed in mature follicles in CT females ($P < 0.05$). However, when considering size, areas were equivalent in F1 and F2 follicles ($P > 0.05$).

Table 1 Area of granulosa layer (mm^2) from secondary, mature and small (F1 and F2) mature follicles in each experimental group

Parameter	CT (n=5)	IM (n=5)	SEM	P value
Secondary	0.14[a]	0.10[a]	0.02	NS
Mature	0.48[a]	0.18[b]	0.05	0.0025
F1 (≤ 1 mm)	0.20 [a]	0.18[a]	0.01	NS
F2 (1.1 – 2.0 mm)	0.40[a]	0.16[a]	0.11	NS

[a,b] Within a row, different superscripts differ ($P<0.05$)

Discussion and Conclusions

In IM gilts, the higher number of less developed follicles, which are gonadotropin-independent, reflects the inhibition of LH and mainly FSH by immunocastration, leading to the absence of larger mature follicles and a greater number of advanced follicles in the final stage of atresia. Moreover, the smaller granulosa layer area (responsible for estrogen synthesis) in mature follicles, and the absence of large mature follicles suggest an inability in estrogen synthesis and secretion and thus, ovarian activity commitment. Therefore, immunocastration of finishing female pigs is effective in inhibiting follicular development and further estrus suppression.

Acknowledgments

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Maximizing Sow Reproduction by Understanding Farrowing Rate when Implementing Ovulation Induction and Fixed-Time AI Protocols

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Introduction

Implementation of single fixed-time AI (SFTAI) without heat detection protocols to manage reproduction in sows is often compromised by failure to correctly evaluate outcomes. Calculation of farrowing rate (FR) by the traditional method has led to misinterpretation of the effectiveness of SFTAI protocols following ovulation synchronization with GnRH analogue products. Some protocols that incorporate ovulation induction followed by SFTAI that include inseminating sows not detected in estrus, are compared to Controls where only sows in estrus are inseminated. Calculating FR by the traditional method (no. farrowed/no. inseminated) does not correctly reflect protocol effectiveness because some sows not detected in estrus do farrow. Thus, the percentage of sows which farrow is greater for those assigned to the synchronized protocol than Controls, even though the traditionally calculated FR is lower. The objective is to propose a metric to better evaluate sow reproductive performance when using SFTAI.

Materials and Methods

Data are summarized from three unique SFTAI protocols utilizing vaginally administered OvuGel® (OG), which contains the GnRH analogue, triptorelin acetate: a fixed-time administration to all weaned sows regardless of estrus (All Sows) or two versions of administration to weaned sows according to signs of estrus (Select Sows) or (Select Sows Plus). In the All Sows Protocol, OG was administered at 96 h post-weaning, without estrus detection. For the Select Sows Protocol, OG was administered only to sows at the early signs of or at first detection of estrus on Day 4 post-weaning. With the Select Sows Plus Protocol, sows detected in proestrus or estrus on Day 4 post-weaning received OG. All sows assigned to OG and Control treatments not detected in estrus received OG on Day 6. Sows administered OG were AI 22 h post-treatment. In all three protocols, non-synchronized Controls were AI at detection of estrus and 24 h later.

Results

Reproductive variables are shown in Tables 1 and 2. The FR is compared as calculated based on sows inseminated, sows in estrus at AI, sows not detected in estrus at AI and for all sows assigned to treatments. The FRs were not different among synchronized sows receiving SFTAI and untreated Controls inseminated each day of estrus when signs of estrus (Select Sows Protocol) are used to identify sows for treatment. However, FRs were lower (All Sows Protocol) for OG-treated sows compared to Controls when treated sows were inseminated without regard to estrus even though more total sows farrow from a breed group. The Select Sows Plus Protocol produces more sows farrowing and live pigs born than the Select Sow Protocol, alone, based on a breed group.

Table 1. All Sows[2] and Select Sows[3] Protocols

	All Sows		Select Sows	
Reproductive Variable	Ctrl	OG	Ctrl	OG
Breed Group (BG)	426	407	1530	1342
FR in Estrus, %	91.6	91.8	86.6	86.2
FR Inseminated, %	91.6^{x}	88.2^{y}	86.6	86.2
FR Not in Estrus, %	NA	39.3	NA	NA
FR Breed Group, %	84.7^{x}	88.2^{y}	84.0	83.8
Live Born/Litter	12.3	12.2	12.3	12.1
LB/100 in BG	1039	1075	1089	1063

^{x,y} Means within same All Sows Protocol row with different superscripts differ numerically ($p < 0.10$).

Table 2. Select Sows Plus Protocol[1]

Reproductive Variable	Ctrl[a]	OG Day 4 Proestrus or Estrus	OG Day 6 Not in Estrus
Breed Group (BG)	301	296	141
FR, %	93.8^{x}	93.4^{x}	42.9^{y}
LB/Litter	13.3^{x}	13.4^{x}	12.2^{y}
LB/100 in BG	1248	1252	523

^{x,y} Means within the same row with different superscripts are different ($p < 0.05$). [a] Sows bred each day of estrus.

Conclusions and Discussion

Farrowing Rate (no. farrowed/no. inseminated) does not reflect effectivness of a SFTAI protocol. More sows farrowed, and thus, more pigs were produced from the All Sows Protocol than the Controls and Select Sows Protocol (Table 1) because "bonus sows", which had silent or missed estrus, conceived, whereas sows from the Controls, which had silent or missed estrus, were not inseminated. Similarly, more sows farrowed and more pigs were produced from the Select Sows Plus Protocol than from Controls (Table 2) because of the "bonus sows" that farrowed from sows not detected in estrus and treated on Day 6. Therefore, to accurately evaluate SFTAI protocols compared to unsynchronized sows, including sows not detected in estrus, the FR should be calculated based on no. farrowed/no. in breed group.

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Tolfenamic acid 4% in gilts: positive effects on litters performance

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Introduction

Postpartum dysgalaxia syndrome (PPDS) is a common and important disorder that affects a swine female, mainly in intensive production systems (2). PPDS is characterized by hypogalactia in the puerperium; its symptoms may vary as fever, appetite reduction, mastitis in the female and signs of hunger in the piglets. In the majority of the cases, hypogalaxia is not clearly identified, assuming a sub-clinical aspect (3). Throughout the last decades, in many countries, tolfenamic acid, a nonsteroidal anti-inflammatory drug (NSAID), has been successfully administered to several animal species, as cats, dogs, cattle, and pigs. Tolfenamic acid is recognized for its analgesic, antipyretic and anti-inflammatory properties (1). The objective of this study was to evaluate the effect of a non-steroidal anti-inflammatory drug (NSAID), based on tolfenamic acid, as a prophylactic treatment of the syndrome of PPDS, on the zootechnical performance of the litters.

Materials e Methods

Gilts (n = 332) were selected and randomly distributed in two treatments groups, n = 157 in treated group and n = 162 in the control group. The treated group received a single intramuscular injection (1 mL / 20 kg of 4% tolfenamic acid (Tolfedine CS CS™) post-farrowing, the control group did not receive any treatment. All piglets (4,466) were weighed at first, fourth and eighteenth day of life. All litter (2,198 and 2,268 piglets for treated and control groups, respectively) were evaluated for weight gain, diarrhea occurrence, piglets and mortality between 4 and 18 days of life. Data were analyzed using the SAS program (2012), differences were considered significant at level of 5% (P <0.05). Piglet´s weight averages were analyzed by covariance considering the effect of the initial weight and the presence of diarrhea. Categorical variables were analyzed by logistic regression. (4)

Results

The treated group had 0.41% less mortality until the 18th day of life (p = 0.0285). This rate increases to 4.5% (4.3% and 8.8% for the treated and control group, respectively) among the litters not affected by diarrhea (p = 0.0005). These results show a positive effect of the treatment, although it loss the benefits if the litter is stricken by diarrhea. Gilts presenting rectal temperature above 39.5°C or mucopurulent vulvar discharge were characterized with (PPDS). Overall, the weight average at 18 days in piglets without diarrhea was higher, 4.949g, compared to 4.593g, than piglets with diarrhea. Specifically for litters without diarrhea, the total of weight gain in the treated group was 9.0% (p <0.05) greater than the control group.

Table 1. Average and standard error of variables analyzed by logistic regression.

VARIABLE	Group		Pr>x²
	Control	Treated	
% PPDS	51.85±3.94	54.14±3.99	0.6449
[1] % D.V. M	16.67±2.94	20.38±3.23	0.3774
[2] % B.T > 39,5 ºC	40.12±3.86	43.31±3.97	0.5378
[3] % P.M at day 18	7.143±0.721	6.733±0.599	0.0285

[1]Discharge vaginal mucopurulent; [2]Body temperature > 39,5 ºC; [3]Piglet mortality at day 18.

Table 2. Effect of diarrhea on piglets and litters performance parameters

DIARRHEA	Group			
	Control	Treated	Average	Pr>x²
	Mortality until 18 day of live			
No	8.851±1.525 [a]	4.348±0.684 [b]	6.599±0.864	0.0005
Yes	6.466±0.801 [b]	7.722±0.782 [a]	7.080±0.561	0.1014
Average	7.143±0.721	6.733±0.599		0,0285
DIARRHEA	**Group**			
	Control	**Treated**	**Average**	**Pr>x²**
	Average weight at 18 th days			
No	4923.4±74.2 [a]	4974.5±73.9 [a]	6.599±0.864	0.0005
Yes	4579.9±51.9 [b]	4607.4±51.8 [b]	7.080±0.561	0.1014
Average	4751.7± 49.3	6.733±0.599		0,0285
DIARRHEA	**Group**			
	Control	**Treated**	**Average**	**Pr>x²**
	Total of weight gain at 18 days			
No	43.99±1.18	48.35±1.17 [a]	46.17±0.83 [a]	0.0086
Yes	42.08±0.82	41.21±0.82 [b]	41.64±0.59 [b]	0.4480
Average	43.04± 0.78	44.78±0.77		0.1093

Average followed by different letters in the same column differ by F or *χ^2 test ($p \leq 0.05$).

Conclusions and Discussion

Tolfenamic acid 4% (Tolfedine CS ™) administered to gilts reduced the piglets mortality during the suckling phase and increased weight gain in litters not affected by diarrhea. The pain and discomfort reduction and in the first days of lactation, contribute to a better litters performance.

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Quality control of the procedures and bacteriological evaluation of boar semen in Brazil studs

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Introduction

In Brazil's current swine production system, the Boar Studs (BS) are the grounds to house boars in order to produce semen doses to attend the sows of nucleus herd, multipliers and commercial farms. The quality of the inseminating dose (ID) used, either by sperm viability or microbiological contamination, may affect the reproductive rates and compromise business profitability (1). The aim of this study was monitoring the procedures, through a check list, and was evaluated the contamination pressures in boar studs.

Materials and Methods

Were measured retrospective data along 24 months (from august/17 to july/19) of 11 Brazilian Boar Studs (BS) located in the states of Rio Grande do Sul (n=3), Santa Catarina (n=5), Paraná (n=1), Mato Grosso do Sul (n=1) and Mato Grosso (n=1), adding up about 1,500 boars (around 20% of boars in the Brazilian squad in production). All units had the same computer semen analysis system (CASA; Magavision® Magapor®, Spain) in use for more than 12 months, calibrated to the supplier's standard, always by the same employee. Periodically, were realized microbiology analysis of raw semen, extended, stored and inseminating doses used in pig farms (reverse analysis). A routine technical visit was carried out. In this visit, the veterinarian, whom is specialized in reproduction issues, evaluated the production flow considering 75 points, there being: boar facility (n=6), boar health (n=8), semen quality analysis (n=5), collection management (n=11), laboratory (n=8), collection procedures (n=23), water quality (n=6) and routine materials (n=8). Each point is given a score (excellent, good, regular or bad). The data were analyzed using Statistix 10. The procedure Kruskal-Wallis Statistic was used and means were compared using Dunn's All-Pairwise Comparisons Test, which sorts the BSs in ranking, however, the results are shown in percentual. Differences were considered significant if $p<0.05$.

Results

There was difference between the BS related to the check list ranking in excellent/good and regular/bad (Table 1). In each auditing visit, a report and an action plan was generated based on the points regular and bad. In raw semen microbiological analysis, only BS X was bounded in 2,000 CFU. The BSs II, III, and V were beyond the limit of 500 CFU for extended semen, stored and reverse analysis (Table 2). The most frequently isolated agents were: *Acinetobacter sp*, *Citrobacter sp*, *Edwardsiella sp*, *Enterobacter sp*, *Escherichia coli, Klebsiella pneumoniae*, *Klebsiella sp*, *Morganella morganii*, *Proteus mirabilis*, *Providencia sp, Pseudomonas aeruginosa, Salmonella sp, Staphylococcus saprophyticus*.

Conclusions and Discussion

The quality of inseminating doses, either in relation to sperm parameters or microbiological, depends on procedures realized in BS.

Table 1. Collection and handling of boar semen process check list in Brazilian studs (Mean±Standard Error Mean).

BS	Visits	Excellent and good %	Regular and bad %
I	9	79.1±2.4 ^{c}	20.9±2.4 ^{a}
II	10	82.9±2.3 ^{bc}	17.1±2.3 ^{ab}
III	12	83.3±2.1 ^{abc}	16.7±2.1 ^{ab}
IV	7	84.0±2.8 ^{abc}	16.0±2.8 ^{abc}
V	21	86.8±1.6 ^{abc}	13.2±1.6 ^{abcd}
VI	14	90.6±2.0 ^{ab}	9.4±1.9 ^{bcd}
VII	11	90.9±2.2 ^{abc}	9.1±2.2 ^{bcd}
VIII	12	91.8±2.1 ^{abc}	8.2±2.1 ^{bcd}
IX	23	93.2±1.5 ^{a}	6.8±1.5 ^{cd}
X	10	95.0±2.3 ^{a}	5.0±2.3 ^{cd}
XI	9	95.8±2.4 ^{a}	4.2±2.4 ^{d}

$^{a, b, c, d}$ In the column differ ($p<0.05$).

Table 2. Bacterial contamination in Colony-Forming Unit (CFU) in raw and extended boar semen (Mean±Standard Error Mean).

BS	Raw	Extended	Stored	Reverse Analysis
I	11001.0±632.6	35.7±30.0	30.8±21.5	
II	6648.1±702.0	321.0±82.2	705.7±121.2	474.2±87.6
III	9810.4±711.5	108.6±51.7	524.8±123.7	840.5±87.9
IV	8484.5±995.5	157.0±156.2	39.1±33.5	60.5±52.5
V	3781.1±541.5	501.7±105.5	180.2±71.6	504.0±41.5
VI	2965.1±526.7	15.7±9.9	140.1±56.0	200.3±54.1
VII	3467.0±473.0	16.1±5.75	268.2±84.7	53.9±32.0
VIII	3467.0±473.0	16.1±5.8	268.2±84.7	53.9±32.0
IX	5868.7±637.8	142.8±54.2	190.8±82.5	420.6±57.6
X	464.3±162.2	47.6±32.9	5.4±3.7	241.5±82.2
XI	4288.7±557.2	105.3±53.3	63.9±40.5	249.9±52.3

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Reproductive disorders following *Leptospira* Pomona infection in a sow farm, results following to antibiotic treatment and vaccination with Porcilis® Ery+Parvo+Lepto

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Introduction

Leptospira Pomona is known as a cause of reproductive failures in sows. Antibiotic treatment was the usual way to handle Leptospirosis in the past. The vaccine availability of Porcilis® Ery+Parvo+Lepto gives us the opportunity to reduce clinical signs and / or infection of nine relevant *Leptospira* serovars.

Materials and Methods

In a 500 sow farm with good reproductive performance (> 30 piglets nursed/sow/year over years; vaccination Parvovirus, Erysipelas and PRRS EU) abortions increased dramatically beginning end of 2017. After detection of Leptospira Pomona (by PCR in abortion material) sows were treated with trimethoprim/ sulfonamide. Despite of improved hygiene precautions and change of antibiotic treatment to doxycycline problems were diminished but did not disappear completely during the next months. With the availability of Porcilis® Ery+Parvo+Lepto vaccination was introduced as 2fold herd vaccination. Incoming gilts were vaccinated 2fold with between 180 and 220 days of age. The sow farm does a proper documentation with a professional sow herd planner (db.planer BHZP).

Results

Herd data (time delayed analysis to farrowing, table 1) showed in the acute clinical phase (Jan-Jun 2018): high numbers of abortions 6.7 %, a strong decreased farrowing rate 80.7 %, also an increase of dead born piglets 9.3% and mummies 0.86%. Results from sows with 2fold doxycycline treatment (Jul-Dez 2018) were improved but stayed clearly behind the previous results before clinical leptospira infection: abortions (3.6%) and dead born piglets 9.3% were still strongly affected, whereas farrowing rate (86.9%) and mummies (0.54%) showed more improvement. Results from sows with 2fold vaccination of Porcilis® Ery+Parvo+Lepto (Jan-Jun 2019) were on the levels and slightly above the performance data before leptospira clinic: abortions had disappeared (0.2%) farrowing rate (85.8%), dead born piglets 7.6%, and mummies (0.47%) were back to normal. Data from the vaccinated period compared to acute period showed 97 % reduction of abortions, an increase of farrowing rate by 6.3 %, 18.2 % less dead born piglets, 45.3% decrease of mummies and 6.23 % more live born piglets/sow/year (40.1 piglets/sow/year to 42.6).

.Table 1. Performance data (time delayed analysis according to farrowing) of the Leptospira Pomona infected sow farm.

	Jan-Jun 2018	Jul-Dez 2018	Jan-Jun 2019	deviation
mating, n	706	642	571	
farrowing rate, %	80.7	86.9	85.8	+6.3%
abortions, n	47	23	1	
abortions, %	6.7	3.6	0.2	-97.4%
piglets live born/litter, n	18.1	18.1	18.7	+3.3%
piglets dead born/litter. n	9.3	9.3	7.6	-18.2%
mummies, n	483	306	267	
mummies /litter, %	0.86	0.54	0.47	-45.3%
litters/sow/ year, n	2.22	2.27	2.28	+0,06
piglets live born /sow/ year, n	40.1	41.1	42.6	+6.2%
piglets weaned /sow/year, n	33.0	33.3	34.7	+5.2%
unproductive days/litter, n	15.2	10.8	7.6	-50.0%

Conclusions and Discussion

Leptospira Pomona is a severe threat for reproduction of sow herds. Antibiotic treatment can reduce clinical symptoms caused by *Leptospira* Pomona but does not solve the problem on a sustained basis.

In the described, particular case vaccination with Porcilis® Ery+Parvo+Lepto resulted in a clear improvement of reproductive performance compared to the previous results during the phase of Leptospira infection. Vaccination with Porcilis® Ery+Parvo+Lepto aids in the protection of sows from *Leptospira* Pomona related reproductive disorders and helps to ensure high health and performance.

Porcine placental histomorphometrical associations with fetal size, gender and gestational age

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Introduction

The availability of a large number of pigs to slaughter at an adequate body weight is essential to obtain the desirable profitability for swine producers. There is evidence that poor fetal growth may give rise to low birth weight piglets, which present compromised postnatal growth performance (1).However, fetal growth in the pig may be affected by placental insufficiency due to intrauterine crowding, leading to the birth of small piglets (2).

Placental weight and efficiency are positively associated to embryo and fetal weights (2). Additionally, there are placental functional alterations due to sexual dimorphism (3,4), as male fetuses have a survival disadvantage compared to females (1,3). Even though there is evidence of placental alterations associated to fetal weight and sexual dimorphism, there is lack of information on placental morphological alterations associating those parameters throughout gestation. Thus, this study aimed to characterize the histomorphometrical changes of the swine placenta during early, mid and late pregnancy, associated to sex and fetal weight.

Materials and methods:

Placental and uterine samples from 17gilts(Large White × Landrace)were collected at gestational ages 30, 45, 60 and 90. These samples belonged tomale (M) and female (F), low (LW) and high weight (HW) conceptuses at gestational days (GD) 30 (n=5 LW and 5 HW), 45 (n=5MHW, 5 FHW, 3MLWand5FLW), 60(n= 3MHW, 3FHW, 2MLW and3FLW) and 90(n=5 MHW, 5FHW, 3MLW, 5 FLW). After processed for histomorphometrical evaluations, the percentage of the endometrial components of the uterine samples (glandular epithelium, glandular lumen, arteries, veins, capillaries and connective tissue)were determined through volumetric proportion. Subsequently, the maternal-fetal interface (length and height of the trophoblast cells and endometrial epithelium cells: thickness of the placental wrinkle) was evaluated inall experimental groups.

Data were analyzed as a randomized design and treatment effects were established by multiple comparisons using the probability of difference (pdiff) between means and $P<0.05$ was considered significant.

Results:

In GD30, embryos were evaluated considering size. In this gestational age, the embryos' size did not affect the uterine components. Interestingly, HW embryos presented numerically higher endometrial epithelium thickness, even though it did not reach the level of significance ($P<0.06$).

Table 1 summarizes the histomorphometrical parameters evaluated in GD 45, 60 and 90. No fetal size effects were observed for the histological parameters evaluated at those specific gestacional ages. On the other hand, at GD 60, a gender effect was observed for trophoblastic cells height, as female fetuses showed greater height compared to males($P<0.05$). Moreover, in GD 90 samples, a gender effect was also observed, as male fetuses showed a larger area between the trophoblast and the endometrial epithelium ($P<0.05$).

Table 1. Histomorphometrical parameters evaluated in male and female conceptuses according to gestational age.

	GD45		GD60		GD90	
	M	F	M	F	M	F
EE	0.9±0.2	0.74±0.2	14.0±2.8^a	28 ± 2.8^b	22.6±2.2	6.8±1.2
TE	21.5±4.7	NA	3.4±0.5	3.6 ± 0.5	8.7±0.8	18.8±3.1
SBE	NA	NA	NA	NA	4.7±0.7^a	1.5±1.0^b

A,b different letters in the same row differ ($P<0.05$) between groups with the same gestational age. M, male; F, woman; EE, endometrial epithelium; TE, trophoblastic epithelium; SBE, area between epithelium; NA, not applicable.

Discussion and Conclusion

Our results suggested that greater thickness of the endometrial epithelium in the HW conceptuses at GD 30 led to a larger contact area between the components of the maternal-fetal interface and, consequently, better nutrition for those embryos. At GD60, the greater trophoblastic epithelium thickness in female fetuses improved the exchange area, thus, placental efficiency. On the other hand, at GD 90, the greater exchange area was observed in male fetuses, which may indicate a compensatory mechanism, since previous studies highlighted that male fetuses have a survival disadvantage compared to females.

It is important to emphasize that the relationship between fetal size, sex and placental components is dynamic and depends on the GD investigated.Thus, at early pregnancy, embryo size determines placental structure. However, as pregnancy progresses, sex is the determinant factor for placental growth. Considering that male fetuses may show a survival disadvantage compared to females, there may be a compensatory mechanism, such as an increase in the nutrient/oxygen exchange area, to benefit their survival.Further studies should be performed to improve the understanding of the mechanisms behind these findings.

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Seasonal Infertility in southeast Brazilian swine farms: Preliminary results

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Introduction

In swine, seasonal infertility has been reported in many countries, including the USA, Canada, Thailand, Japan, Australia and others European countries (1,2). The *main causes* of seasonal infertility identified are high temperatures alone or in combination with humidity and longer photoperiods during summer (2,3,4). Major manifestations of seasonal infertility include delayed puberty, increased returns to estrus rate (RE), extended wean to estrus intervals, pregnancy loss, reduced farrowing rate and lower litter size (2). However, these problems may not be observed all at once, and may vary widely among herds, year, housing system, and in relation to other management and environmental factors (2,4). In Brazil, the incidence of seasonal infertility has not been fully evaluated. Thus, the present study aimed to determine if the pattern of RE as an indicator of seasonal infertility reported in many studies elsewhere, is also evident in commercial pig herds in the southeast region of Brazil.

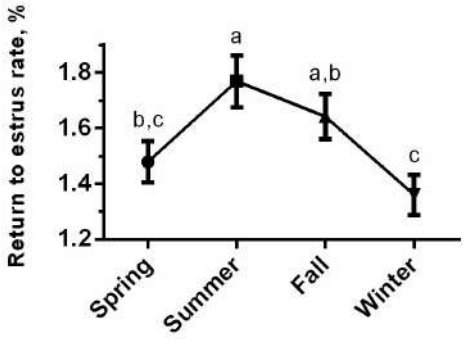
Materials and Methods

The present study was a retrospective analysis using data records from the Agriness® S2 software (Agriness, Florianópolis, SC, Brazil). The data was obtained from four selected commercial herds with crossbred Landrace and Large White females (DB Swine Genetic, Patos de Minas, MG, Brazil). The herd sizes were 1600, 1800, 2500 and 3000 sows, with gestation housing in collective pens and individual crates. The dataset included weekly records of 86,080 artificial inseminations (AI) and RE from January 2015 to December 2019. The years were divided into four seasonal periods: Summer, from weeks 1 to 11 and > 50; Fall, from weeks 12 to 24; Winter, from weeks 25 to 37; Spring, from weeks 38 to 50. Statistical analyses were conducted using SAS version 9.4. A generalized linear model was used to test the binary response variable (weekly RE number / AI number) using the GLIMMIX procedure with season, year as main effects and their interaction. Crossbreds and herds were used as random effects.

Results and Discussion

There was no interaction of season with year for RE. Higher values of RE were observed in Summer and Fall compared with Winter (Figure 1). Further, RE was lower in Spring compared with Summer, however, no differences were found when Spring was compared to Winter or Fall. Our results confirm a seasonal effect on increasing RE is evident in the southeast region of Brazil. Seasonal infertility involves multifactorial causes such as heat stress, long photoperiod or their combination. Their effects are related to alterations in gonadotrophic stimulation, such as lower GnRH release and an attenuated preovulatory LH surge in sows (2,3). In addition, attenuated kisspeptin production may occur as a result of down-regulated KiSS-1 gene expression and/or to the negative effects on follicle development by changes in pattern of melatonin secretion (5,6). In the present study, there was an observed year effect, with the RE values decreased by 1.97, 1.99, 1.75, 1.21 and 0.90% from 2015 to 2019, respectively. These observations may be related to Genetic improvement and environmental changes from year to year.

Figure 1. Return to estrus rate by season



[abcd]Mean values with different letters differ (P<0.05); interaction season and years P>0.05

Conclusions

Measuring RE can be used an indicator of seasonal infertility in the southeast region of Brazil. More studies are necessary to better understand the seasonal effects according to weather indicators as temperature and photoperiod in that region.

Acknowledgments

To DB Swine Genetic and FAPESP process 2019/02113-3.

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Effect of parity order on the reproductive performance of sows raised in a tropical environment

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Introduction

The parity order of swine matrices interferes with its reproductive performance. Females from third to fourth parity order have the best reproductive rates, and a decline after this peak, this may occur due to the physiological factors of the females and selection on a farm (1). Primiparous have a litter size smaller than sows in the third and fourth parity order (1). In temperate environment, the sow has low reproductive performance under heat stress, occurring mainly in the summer (2). The objective of this study was to verify the interference of the parity order in the reproductive performance of commercial swine raised in a tropical region.

Materials and Methods

The study was carried out from September 2013 to July 2019 on a commercial farm located in the Brazilian Cerrado (18º 91 'S, 48º 25' W and 875 m altitude) in the mesoregion of Triângulo Mineiro, Minas Gerais, Brazil. The climate is tropical with dry winter and Aw type according to the Köppen climate classification (3). The meteorological data of a station were analyzed during the study period, recording the days of occurrence of heat waves, considering three or more consecutive days with temperature $\geq$ 25 ° C and Temperature and Humidity Index (ITU) > 74. The performance data of the farm animals were recorded in the Agriness S2 software with information from 12,491 matrices and 51,578 inseminations. The effect of the parity on reproductive performance was verified, sorting out the females into categories: 0 (gilts), 1 (parity 1 sows), 2 (parity 2 to 5 sows), 3 (parity $\geq$ 6 or more sows). Reproductive data were analyzed using the Statistical Analysis System (SAS) program. The data did not meet the assumptions of normality and homogeneity of variance and the Kruskal - Wallis nonparametric test was used. A significance level of 5% (P <0.05).

Results

The total of 2,156 days evaluated, there were 1,163 days of heat waves. The gilts had a lower number of live born, stillborn, dead at birth, mummified piglets and lighter litters in relation to sows from two to five parities. Primiparous had a lower number of stillborn compared to the other categories. The females from second to fifth parity order had a higher number of piglets born alive and a higher litter weight. Sows with six or more parities have a lower number of live born piglets, a higher number of stillborn, deaths at birth, mummified and lower weight of the litter compared to the other categories.

Conclusions and Discussion

Sows in a tropical environment showed a productivity pattern similar to those in a temperate environment, and females with six or more parities have a higher risk of stillborn piglets, regardless of temperature (4). The worst reproductive performance in sows with 6 or more parities reveals the importance of replacement in a farm. The gilts and primiparous are not yet at their best performance, but the introduction of these females in the herd and the proper management contribute to obtaining desirable reproductive index.

Table 1. Averages (standard deviations) of the reproductive variables of the sows of commercial farm of different categories in a tropical environment

Category *	Live born piglets (n)	Stillborn (n)	Mummified (n)	Litter weight (kg)
0	11.84^{c} (3.16)	0.83^{b} (1.18)	0.29^{a} (0.71)	16.26^{c} (4.45)
1	11.95^{b} (3.27)	0.66^{a} (1.04)	0.30^{a} (0.70)	16.48^{b} (4.63)
2	12.36^{a} (3.21)	0.89^{c} (1.24)	0.35^{b} (0.72)	17.02^{a} (4.54)
3	11.38^{d} (3.23)	1.07^{d} (1.42)	0.40^{c} (0.79)	15.36^{d} (4.47)

Average followed by different letters in the same column differ from each other by the Kruskal - Wallis test (P <0.05). * Categories: 0 (gilts, n = 9,651), 1 (first parity, n = 8,451), 2 (2-5 parity order, n = 23,000), 3 ($\geq$ 6 parity order, n = 8,001)

Parity 2 to 5 sows have the best reproductive rates, being desirable in a commercial farm, but sows with six or more parities, reproductive performance decreases. These results justify the important to select and replace the sows in a commercial farm, in addition to controlling temperature and humidity in a tropical environment, to avoid low reproductive performance.

Acknowledgments

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Effects of deoxynivalenol and/or fumonisin B_1 on ovaries of pigs

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Introduction

Mycotoxins are natural contaminants of food and feed occurring worldwide. Among these toxins, deoxynivalenol (DON), and fumonisin B_1 (FB_1) have been considered a major problem for the pig health. After the ingestion of DON and FB_1 contaminated feed, intestinal and immunological changes contribute to a poor growth performance (1). Moreover, during the last years, the reproductive effects of DON and FB_1 have been suggested by previous studies. However, studies on reproductive toxicity caused by DON and FB1 in swine are scarce and mainly based on *in vitro* models (2). Thereby the present study has the objective to evaluate the effects of DON and FB_1, in combination or alone, on the ovarian morphology and oxidative stress response using porcine explants.

Materials and Methods

Seventy-two explants were obtained from six pigs and submitted to the following treatments: control (MEM medium), DON (10 µM), FB_1 (100 µM FB_1) and DON+FB_1 (10 µM+100 µM). Histological changes were evaluated using a lesional score. In addition, the number of normal and degenerated follicles at all stages of development (primordial, primary, and growing) was evaluated. Staining of nucleolar organizing regions (NORs) with colloidal silver nitrate was used the evaluation of granulosa cell proliferation, whereas the apoptosis was evaluated using an imuno-histochemical method with the antibody anti-caspase 3. The oxidative stress response was performed taking into consideration the lipid peroxidation (thiobarbituric acid-reactive substances-TBARS) and antioxidant capacity response assays (reduced glutathione - GSH, ferric reducing antioxidant power - FRAP, and free radical scavenging capacity - ABTS).

Results

DON and/or FB_1 induced significant histological changes accompanied by a decrease in the number of viable follicles and an increase of degenerated follicles at all stages of development in relation to the control group ($P \leq 0.05$). In addition, explants exposed to DON and/or FB_1 showed a significant decrease in granulosa cell proliferation ($P \leq 0.05$). The individual treatment of explants with DON and FB_1 induced no significant changes in cell apoptosis of primordial and primary follicles. However, a significant increase was observed in the number of apoptotic cells of growing follicles exposed to combined treatment of DON plus FB_1 compared to control ($P \leq 0.05$). DON induced no change on ovarian oxidative stress response, whereas in the explants exposed to FB_1 and DON plus FB_1 a significant decrease in the lipid peroxidation was accompanied by a significant increase in the antioxidant response ($P \leq 0.05$).

Conclusions and Discussion

The continuous ingestion of low doses of mycotoxins is a reality for animals, however, the impact on the reproductive system is scarcely known. In the present study, we have demonstrated that DON and/plus FB1 exposure induced histological changes in the ovaries of pigs, including alterations in cell proliferation and apoptosis. Similar results were observed previously in ovarian explants of pigs exposed to DON (3). In contrast, rats fed an FB1-contaminated diet showed no histological abnormalities in the ovaries, although a significant reduction on the serum FSH and LH levels has been observed (4). Pigs are the animal species most suceptible to the effects of mycotoxins, and this may explain the different results (5). Modulation of the oxidative stress response was also detected in the explants exposed to FB_1, and DON plus FB_1. Considering that reactive oxygen species can play na important physiological role in the ovaries (6), the increase observed in the antioxidant potential induced by the treatments containing FB_1 may affect the reproductive capacity in pigs. In this context, future studies should be designed to characterize the role of ROS in the reproductive toxicity of mycotoxins in an *in vivo* model.

Acknowledgments

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Birth weight impacts on testicular morphology and steroidogenesis in adult boars

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Introduction

To increase herds' productivity, breeding programs have focused on reproductive efficiency by increasing gilts' ovulation rate. However, the rise in the number of piglets weaned per female is not followed by an increase in uterine blood flow, which could affect nutrients and oxygen supply, resulting on the birth of low weight piglets, which would not reach their full growth potential (1, 2). Nonetheless, little is known about the impact of birth weight on reproductive traits and performance in boars.

As artificial insemination (AI) is a facilitating global improvement in fertility and genetics dissemination and low birthweight piglets are a reality at commercial farms, it seems necessary to better understand the mechanisms that govern it. Hence, the objective of this study was to investigate testicular morphology, and the steroidogenic process in different birthweight boars.

Materials and Methods

Twenty-four newborn littermate boars were selected after birth and divided into two experimental groups, according to birthweight. High (HW), ranging from 1.80 to 2.25kg (n=12) and Low (LW), ranging from 0.75 to 1.10kg (n=12). At 300 days of age, 7 males from each experimental group were orchiectomized and 5-mL blood samples were withdrawn from the jugular vein for plasma testosterone concentrations assessment (electrochemiluminescence immunoassay - ECLIA).

Additionally, testicular fragments were collected and processed for further histomorphometrical analyses such as measurements of the seminiferous tubule diameter and seminiferous epithelium height, and the volumetric proportion of the testicular components, using a 441-point grid place in an eyepiece of the light microscope. Moreover, the relative expression of the steroidogenic enzyme 17α-hydroxylase (17α-OH) was measured through qPCR, and androgen receptors were quantified by immunofluorescence in Leydig cells. Treatment effects were established by ANOVA and comparisons between means done by the Student T test.

Results

Higher testicular weights and volumes were observed in HW animals ($P<0.05$ – Table 1). However, birthweight did not affect the seminiferous tubule diameter, epithelium height, 17α-OH mRNA relative expression, neither testosterone plasmatic levels. In contrast, LW animals presented lower expression of androgen receptors in Leydig cells and shorter seminiferous tubule length ($P<0.05$ – Table 1).

Table 1. Biometrical, histomorphometrical, hormonal and molecular data of 300-day-old low (LW) and high (HW) birthweight littermate boars.

Parameter	HW (n=7)	LW (n=7)
Bodyweight (kg)		
At birth	1.9 ± 0.4^a	0.9 ± 0.3^b
At castration	199 ± 3.6^a	168 ± 4.4^b
Testis weight (g)	458 ± 17^a	352 ± 21^b
Testis volume (cm^3)	546 ± 54^a	727 ± 45^b
Tubule diameter (μm)	262 ± 4.3^a	260 ± 5.1^a
Total length of seminiferous tubule (m)	7323 ± 348^a	6093 ± 376^b
Seminiferous epithelium height (μm)	92 ± 1.2^a	95 ± 1.4^a
Relative expression 17α-OH	4.0 ± 0.9^a	1.9 ± 0.6^a
Testosterone ng/ml	4.2 ± 0.9^a	3.9 ± 1.1^a
Androgen Receptors	8.5 ± 0.03^a	5.6 ± 1.1^b

[a,b] Within a row, different superscripts differ ($P<0.05$)

Discussion and Conclusions

The biometrical data showed that LW animals present smaller testis, without any pathological conditions. The shorter seminiferous tubule length reflects a sperm production commitment in those males. Knowing that testosterone plays an important role on sperm production, it was initially expected that the LW group would present lower testosterone concentrations. Surprisingly, birth weight did not alter testosterone levels nor steroidogenesis, even though there were less androgen receptors in LW males. Hence, HW boars have the potential to produce more spermatozoa and consequently more semen doses per ejaculate, and would be very valuable to an industry that relies on AI.

Acknowledgments

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Reproductive performance in Spain can be successfully predicted

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Introduction

In a previous study (De Andrés et al., (2019) it was presented the evolution of reproductive performance in Spanish farms in the last 10 years, including the main KPIs such as total piglets born (TB), piglets born alive (BA) and farrowing rate (FR). Besides, the same authors predicted the performance for 2019 of this main KPIs (De Andrés et al., (2019). The BDporc® System is a service for the Spanish pig sector. One of its main objectives is to provide reference information to pig production companies using their database.

This study aimed to calculate the error rate in the main KPIs between the predicted values so far and the real data obtained with the official publication of BDporc for 2019 as well as to evaluate the efficiency of prediction models.

Materials and Methods

To evaluate the reproductive performance in Spanish farms during the last 10 years data from 260 farms and a total of 255,386 sows were obtained from the PigCHAMP Pro Europa SL database in the interval 2009-2018. Time series analysis was performed by R software. The prediction of productive performance from October 2018 to September 2019 was performed using Autoregressive Integrated Moving Average (ARIMA) model for forecasting.

The real data were taken from BDporc database for the same period (October 2018 - September 2019). The relative error was used to calculate error metrics for time series forecast and compare predicted values with real ones.

Results

The results of predictions and real data are shown in Table 1.

Every real data provided by BDporc® were within the confidence band of prediction obtained by the time series model of each KPI. The relative error of each prediction was: 1.25% for TB corresponding to 0.20 piglets TB; 0.84% for BA which correspond to 0.12 piglets BA and, finally, considering the FR the relative error was 0.96% corresponding to 0.82 of difference in the percentage of the FR.

Table 1. Real data and predictions of the main KPIs obtained from BDporc and PigCHAMP Pro Europa databases, respectively.

	Real data	Prediction	Confidence[1]	Error[2] (%)
TB	15.63	15.83	(15.55-16.10)	1.25
BA	14.29	14.17	(13.91-14.43)	0.84
FR	85.43	84.61	(82.93-86.28)	0.96

TB: tota born; BA: born alive; FR: farrowing rate.
[1] Confidence bands of prediction.
[2] Relative error

Discussion and conclusions

The results of the present study show that the ARIMA model works very good as a model for forecasting since the prediction models of time series obtained a high hit rate (every real data was within confidence bands of prediction). Moreover, the relative errors were minimal, being 1.25% the highest one.

Therefore, it is concluded that the built models can predict the upcoming years' performance for these KPIs with a high accuracy and success rate.

May also to consider that new technologies and innovative equipment of farms allow massive data collection which properly used can help to optimise production. This study encourages the collection of reliable and quality data from every aspect of production. Moreover, the combination of reproductive, environmental or feed intake data helps to predict farm production. It is important to keep in mind that as existing data from farms or companies, many times underused can predict or anticipate events and therefore improve the decission-making process, improving the competitiveness of the company.

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Molecular characterization of Leydig cell populations in postpubertal pig testis

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Introduction

Leydig cells, present in the intertubular compartment of the testis, are the main source for the sexual steroids hormones. In this regard, the balance between androgen and estrogen is especially necessary for maintaining a successful spermatogenesis. In pigs, two morphologically distinct Leydig cells types are recognized within the interstitial compartment and they seem to present a preferential distribution, since a hypertrophied cell type is observed in the intermediate region (ID) and, showing a small individual volume, a second cell type is frequent in the peripheral region (RP) of testis parenchyma. Despite the morphometrical parameters (1), no other evaluation regarding the functional characteristics of these cells was performed. Therefore, the present study aimed to characterize the differential expression of androgen (AR), estrogen (ERα) and luteinizing hormone receptors (LHR), as well as the proliferation index, in order to better understand the functional differences that may have between these two cell populations.

Materials and Methods

ID and RP representative fragments were obtained from testes of postpubertal pigs (n=4), fixed and prepared for immunohistochemistry evaluation. Primaries antibodies against LHR, AR, ERα and, the proliferation marker, Ki67, were used. The intensity of labeling for LHR, AR and ERα was performed using ImageJ software as described elsewere (2). Calculation of the proliferation index was based on the number of Ki67 positive cells per 1000 Leydig cells. The quantitative parameters were tested regarding the normality condition and for the parametric data one-way ANOVA model, followed by t test, was used. Kruskall-Wallis followed by Mann-Whtiney posthoc were applied for the non-parametric data (GraphPad Prism version 6.01 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com). The level of significance considered was $p<0.05$.

Results

Leydig cells in RP presented a weaker intensity of labeling for ERα when compared with ID cells ($p<0.05$). However, no differences related with AR and LHR immunoreactivities were observed between regions (Figure 1).

Regarding the index of proliferation (Figure 2), RP was characterized with a higher number of Leydig cells presenting proliferative activity (13.8 ± 2.35) in comparison with ID (6.1 ± 1.61).

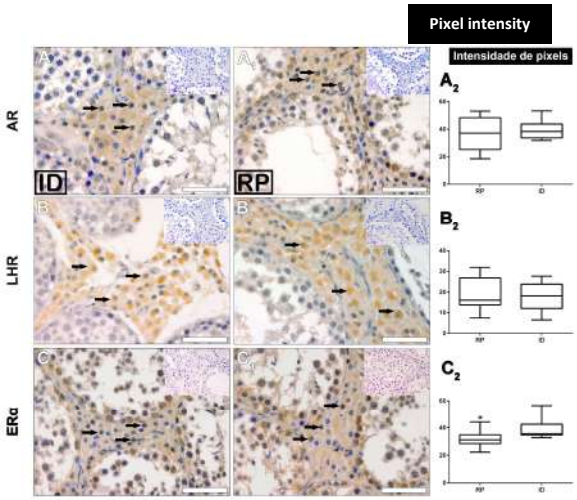

Figure 1: Photomicrographs of intermediate (ID; A, B, C) and peripheral regions (RP; A1, B1, C1) of testicular parenchyma from postpubertal pigs, presenting immunolabeling for androgen (AR), LH (LHR) and estrogen (ERα) receptors and, intensity of labeling (A2, B2, C2). Black arrows: labeled Leydig cell; Inserts, negative control. Bar = 50 μm.

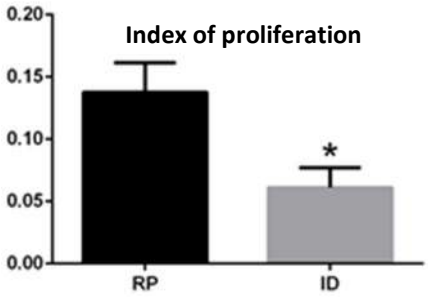

Figure 2: Index of proliferation. Although observed in both regions, Leydig cells Ki67+ number was smaller in ID ($p<0.05$).

Conclusions and Discussion

Based on the proliferation index, we suggest that RP may be gathering a number of progenitors and immature Leydig cell, whereas ID has maintained the mature type. Also, estrogens apparently are playing a specific role on Leydig cells from ID since the labeling of ERα was more intense in this region. However, further studies are being developed, so, we will be able to comprehend the cells functions and how they modulate the different regions of testis parenchyma.

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Factors associated with farrowing rate in commercial pig farms in two consecutive years

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Introduction

A reduced farrowing rate (FR) indicates reproductive failure, increasing replacement costs (4). The studies that seek to associate management, facilities and feeding with reproductive performance of sow generally assess a limited set of factors or focus on production conditions in European (1) or North America countries (6). The aim of this study was to identify and quantify the effects of factors related to performance, management, facilities, feeding, health, and biosafety on FR in Brazilian pig farms in two consecutive years (2014 and 2015).

Materials and Methods

Data from 150 farms were collected (totalizing 135,168 sows). The farms were located in the South (42%), Southeast (45%) and Centre-West (13%) regions of Brazil. A questionnaire focusing on reproductive performance (for the years 2014 and 2015), management, facilities, feeding, health, and biosafety was administered. The questions included replacement, mating, gestation and farrowing/lactation phases. Farrowing rate for the years 2014 (FR-2014) and 2015 (FR-2015) were selected as the dependent variables, corresponded to the mean values from 1 January to 31 December for each year. Farms with a non-stabilized breeding herd were excluded. In addition, not all farms had computed FR data. Thus, we analyze 134 farms in 2014 and 137 in 2015. The farm was considered as the experimental unit. Independent variables (factors) with $p \leq 0.10$ for the F-test in the univariate linear regression model were selected and subjected to Pearson´s and Spearman´s correlation analysis. When the correlation coefficient was high (≥0.60), only one variable was kept. The remaining explanatory variables were included as fixed effects in multiple linear regression models and subjected to a manual forward selection to select those explanatory variables significantly ($p < 0.05$) associated with the dependent variables. Interactions between factors were analyzed.

Results

The averages FR-2014 was 89.83 ± 2.97% (ranging from 82.64 to 96.4%) in 2014, and FR-2015 was 90.07 ± 3.21% (ranging from 81.74 to 96.68%). Lower FR-2014 and FR-2015 were found as abortions and total sow mortality increased (Table 1).

FR-2014 was better in farms that fed pregnant sows with liquid/soup. Farms with higher FR-2015 had wet-dry feeders and practiced all-in all-out management in farrowing rooms.

Table 1. Effects of production factors on the FR-2014 (%) and FR-2015 (%)

Variable	Category	Estimate (SEM)
FR-2014		
Intercept	-	95.97 (0.93)***
Abortion (%)	-	-0.76 (0.25)**
Total SM (%)	-	-0.25 (0.07)***
Physical form of feed for GS	Mash	-3.58 (0.77)***
	Pellet	-2.86 (0.96)**
	Liquid/soup	0
FR-2015		
Intercept	-	92.30 (0.87)***
Abortion (%)	-	-1.04 (0.25)***
Total SM (%)	-	-0.24 (0.07)**
Type of drinker in FR	Wet-dry	1.77 (0.61)**
	Water cup	0.95 (0.70)
	Nipple	0
Housing system in FR	All-in all-out	1.20 (0.60)*
	Continuous	0

SM= sow mortality; GS= gestating sow; FR= farrowing room. *p <0.05; **p <0.01; ***p <0.001.

Conclusions and Discussion

The increase in abortion consequently decreases FR. In addition, abortions may indicate that there are infectious reproductive problems on the farm. For mortality of sows, assuming that a sow spends about 75% of her life in gestation, it is highly plausible to consider that the death reduces FR. Regarding feeding, mash or pelleted diets with a high content of fine particles can cause lesions gastric (5), favoring a scenario of reproductive failure. Otherwise, wet-dry feeders allows the sow to decide the extent to which they want to mix water with food, increasing food intake and reducing the loss of body weight (2). All-in all-out management provides a simultaneous and homogeneous reduction in the level of contamination in all farrowing pens (3), favoring an improvement in FR. Several factors affect FR, but in general these factors do not remain the same over the years.

Acknowledgments

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VIRAL DISEASES

Porcine circovirus type 2 (PCV-2) in southeastern Brazil (from 1967 to 2018)

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Introduction

Porcine circovirus type 2 (PCV-2) is responsible for major economic losses in various parts of the world due to higher mortality, weight loss and low food conversion [1]. The remarkable genetic variability and peculiar evolutionary pathways of the virus is related to its geographical distribution and its globalized presence linked to livestock movements and trade routes lead to the rapid spread of new strains in several countries [2].The aim of this study was to carry out through a retrospective study of the virus based on molecular characterization of PCV-2 in tissue samples obtained from 1967 to 2018 in southeastern BRAZIL (Espírito Santo and Rio de Janeiro States).

Materials & Methods

Swine tissues were obtained from two different origins: frozen tissue from 35 slaughtered pigs and Formalin-Fixed Paraffin-Embedded (FFPE) fragments of tissues samples from 143 pigs from Southeastern Brazil obtained in the last 51 years (1967 to 2018) . FFPE tissue , having different clinical signs, submitted to necropsy from 1967 to 2016 in Rio de Janeiro were provided by PESAGRO-RIO. Slices from each FFPE blocks were pooled by animal and a Deparaffinization Solution QIAGEN® was used for deparaffinization followed by DNA extraction with commercial QIAMP DNA FFPE Tissue Kit® according to manufacturer instructions. Frozen samples (Lung, kidney, liver and lymph node) from 35 pigs were collected at slaughterhouse in the state of Espírito Santo in 2017 and 2018. Tissue fragments were stored at -20ºC until processing, pooled by animal, identified and the DNA extraction was performed as described by Boom et al.[3]. FFPE tissues from 143 pigs and frozen tissue from 35 pigs were tested by Nested PCR targeting a 225 bp fragment of the PCV-2 capsid gene as described by Kim et al [4].

Results

A total of 39/178 (21.9%) samples tested positive for PCV-2. Of these, 9/35 (25%) were frozen samples and 30/143 (20.9%) from FFPE samples. The sample RJ018/1967 is the oldest positive sample for PCV-2 in Brazil to date.

Discussion and Conclusions

One of the biggest challenges of this study was the use of archival swine tissue stored for up to 50 years in the molecular diagnosis and characterization of PCV-2 .Tissue treatment with 10% formaldehyde results in DNA fragmentation resulting in false negative results by degradation of target nucleic acid and / or presence of substances that could inhibit the PCR reaction [5;6]. In this study, PCV-2 was detected in one sample from 1967, which was the oldest FFPE tissue fragment available in the PESAGRO-RIO collection, the second oldest positive sample in the world and the oldest in the Americas. These results corroborate a retrospective study suggesting that PCV-2 has possibly caused isolated episodes of systemic disease since 1962, long before the major epidemic from the 1990s [7]. In conclusion, the technique of DNA extraction in ancient tissue made it possible to obtain PCV-2 genetic material from FFPE samples up to 50 years, expanding the information about the evolution of PCV-2 in Brazil

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Retrospective study of porcine circovirus type 3 in southeastern BRAZIL (1967-2018)

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Introduction

Porcine circovirus type 3 (PCV-3) was recently described in word [1,2] and it's importance in swine herds in Brazil is still under investigation. The aim of this study was to carry out through a retrospective study of the virus based on molecular characterization of PCV-3 in tissue samples obtained from 1967 to 2018 in southeastern Brazil.

Materials & Methods

Swine tissues were obtained from two different origins: frozen tissue from 35 slaughtered pigs and Formalin-Fixed Paraffin-Embedded (FFPE) tissues samples from 143 pigs. FFPE, obteined from 1967 to 2016, were kindly provided by PESAGRO-RIO. Slices from each FFPE blocks were pooled by animal and DNA extraction performed with commercial QIAMP DNA FFPE Tissue Kit®. Frozen samples (Lung, kidney, liver and lymph node) from 35 pigs were collected at slaughterhouse in 2017 and 2018. The extraction was performed as described by Boom et al. (1999) [3]. Nested PCR was performed targeting a 203 bp fragment capsid gene of PCV-3 [4].

Results

A total of 26/178 samples (14.6%) tested positive for PCV-3: 14/35 (40%) frozen tissue and 12/143 (8,4%) FFPE tissues. PCV-3 was detected in the 1960s, 1970s, 2000s and 2010s with the molecular characterization of two genotypes PCV-3a and PCV-3b.

Discussion and Conclusions

PCV-3 DNA has been detected over the 51 years of study in both sources, and the FFPE sample detected in 1967 (RJ10/1967) is so far the oldest PCV-3 partial capsid sequence ever described. One of the main challenges of this study was the use of swine FFPE tissue samples since DNA extraction from ancient FFPE blocks has some critical points that may culminate in PCR amplification failure [5]. PCV-3 was first reported in Brazil by Tochetto et al. [6] in swine serum pools collected in 2017. The first retrospective study was performed by Saraiva et al [7] in Brazil with DNA extracted from swine tissue collected between 2006 and 2007. The detection of PCV-3 in Brazil in 1967 (present study) indicates that the virus circulated in swine populations decades before initial reports, whether or not it was associated with clinical manifestation.

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Commercial feed containing porcine plasma spiked with *African Swine Fever Virus* is not infective in pigs when administered for 14 consecutive days

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Introduction:
African swine fever virus (ASFV) is an enveloped dsDNA virus that can cause high mortality in pigs of all ages. ASFV is primarily transmitted by oro-nasal route or from ingestion of pork or other contaminated products (swill, waste, carcasses, etc.).
A recent study (1) calculated that 10^4 $TCID_{50}$ was the minimum infectious dose and repeated exposures to feed infected with ASFV increased the probability of infection. The objective of this study was to assess if commercially collected liquid porcine plasma inoculated with ASFV and mixed with commercial feed administered for 14 consecutive days was capable of infecting pigs.

Materials and methods:
A total of 2.8 liters of liquid porcine plasma were divided in 2 batches of 1.4 liters per batch. One batch was inoculated with ASFV (Georgia 2007 strain) to achieve a final titer of 3.4 log $TCID_{50}$/mL. Virus was titrated by immuno-peroxidase test (IPT) on alveolar macrophages. The remaining batch was not inoculated with ASFV and served as a negative control. To prepare inoculum in feed, 100 mL of inoculated plasma were manually mixed and shaken with 1000 g of feed. The mixture was prepared daily 24 h before administration and stored at 4°C to allow for complete absorption of liquid into the feed.
Twenty 4-5 week-old pigs were divided in 2 groups of 10 pigs each, which were allocated in 2 separate rooms at CReSA´s biosecurity level 3 facility to avoid cross contamination between groups. Pigs from the positive control group were fed daily an average of 110 g of ASFV-inoculated feed for 14 days (4.4 log daily exposure/pig). Feed was always administered after overnight food deprivation to ensure pigs ingested all infected feed. The negative control group received an average of 100 g of feed mixed with 10 mL of plasma without virus per pig every day for 14 days. Both groups received additional control feed increasing from 200 to 400 g/pig each day to assure proper nutrition. Both groups were normally fed 500 g/pig/day from day 19 to 28. On day 19 of the study, 2 pigs from each group were euthanized and necropsied. On day 20, 4 pigs that had previously received the ASFV inoculated feed were inoculated by intragastric tube (IT) with 50 mL of the same plasma spiked with ASFV, and the other 4 pigs from this group were injected with 10 mL of the ASFV inoculated plasma intramuscularly (IM).The same procedure was done for the negative control group, but in this case using non-inoculated plasma. Pigs were observed for clinical signs daily for 8 days after IM or IT administration. Samples of blood and body temperatures were taken at days 0, 2, 4, 7, 9, 11, 14, 19, 22 and 28. Blood was analyzed by qPCR to detect viremia and sera was analyzed by ELISA to detect ASFV-specific antibodies. At necropsy, samples of tonsil, spleen, and retropharynx, submaxilar and gastrohepatic lymph nodes were taken for qPCR analysis.

Results and discussion:
Up to day 19, all pigs that received ASFV inoculated plasma in feed (4.4 log $TCID_{50}$/d) remained asymptomatic, seronegative and without viremia during the entire study, with no gross lesions at necropsy and no ASFV found in the collected tissues. However, all 4 pigs that received plasma inoculated with ASFV by IM injection (day 20), developed viremia at day 3 post-injection (dpi) and had gross lesions at necropsy with high loads of ASFV (low Ct values) in the tissues. Pigs that received ASFV inoculated plasma (50 mL) by IT developed viremia at 7-8 dpi, had gross lesions at necropsy, and tissues also had high loads of ASFV. As expected, the negative control group remained free from ASFV. Both groups did not seroconvert by ELISA (Ingenzim PPA Compac K3, Ingenasa, Madrid) against the virus throughout the study.

Conclusion:
Pigs exposed to feed containing 4.4 log $TCID_{50}$/d ASFV and consumed daily for 14 days of repeated exposures was not infective, suggesting the potential infectious dose of ASFV in feed is much higher than previously reported, at least when using ASFV-spiked plasma.

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Seroprevalence of porcine circovirius type 2 infection in the area of Vojvodina

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Introduction

Porcine circovirus type 2 (PCV2) is one of the most common pathogens in pig population worldwide, causing significant economic losses in commercial production. Based on previous scientific information, for PCV2 virus-infected pigs there is no unique and final definition of the clinical picture and the disease because all of their most important virulence factors are not yet fully known and analyzed. PCV associated diseases have many different clinical manifestations. The Republic of Serbia belongs to countries that have reported presence of PMWS, but there is no sufficient data to establish the importance of the disease in their pig populations (1). The aim of this research was to determine seroprevalence of infection caused by PCV2 in different categories of pigs on the territory of the province of Vojvodina.

Materials and Methods

The research was conducted on 6 pig farms placed in different locations on the territory of Vojvodina (two farms from the territory of Bačka, Banat, and Srem, respectively) and ranging in the capacity from 500 to 2500 sows, with intensive management and closed type. Blood samples from brachiocephalic plexus were collected from 540 pigs in three different production categories. Specific PCV2 antibodies were determined using indirect immunoenzyme test - INGEZIM CIRCO IgG (Ingenasa, Spain). Performing of the ELISA assayand interpretation of the results was made according to manufacturer's instructions.

Results and Discussion

The percentage of positive animals in all 540 samples collected on the territory of the provice of Vojvodina is 74.8%. The highest percentage of positive animals is in the category of fattener pigs (86.0%) and the lowest in the category of weaned piglets (65.3%), while there were 74.5% of positive specimens in the category of suckling piglets. (Table 1).

Results from our study indicate that the highest percentage of positive animals is in the category of fattening pigs with 86.0%, followed by suckling piglets with 74.5%, and the lowest percentage of positive animals is in the category of weaned piglets with 65.3%. High level of maternal antibodies taken with colostrum can explain high percentage of piglets positive to specific PCV2 antibodies. Estimated half-life of PCV2 antibodies is 19 days, and level of passively received immunoglobulins goes under ELISA "cut off" levelat the age of 5 weeks (2), providing sufficient time for the significant loss of the passively transferred maternal antibodies via colostrum.

Table 1. Seroprevalence of PCV2 in different pig categories in the territory of Vojvodina.

Pig category	Negative	Positive	Total
Suckling piglets	56 (25.5%)	164 (74.5%)	220 (100%)
Weaned piglets	59 (34.7%)	111 (65.3%)	170 (100%)
Fattening pigs	21 (14.0%)	129 (86.0%)	150 (100%)
Total	136 (25.2%)	404 (74.8%)	540 (100%)

Conclusions

Based on our results, we can conclude that PCV2 infections are widespread on the territory of Vojvodina, with the total seroprevalence of 74.8%. The highest value of seroprevalnce is determined in the category of fattening pigs with 86.0%, followed by the category of suckling piglets with 74.5%, and the lowest seroprevalence is in the category of weaned piglets with 65.3%.

Acknowledgments

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African swine fever in Vietnam: What could be learned for the world?

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Introduction

First African swine fever (ASF) outbreak in Vietnam was officially announced in February 2019 and just 6 months later, in august 2019, the disease has spreading all of 63/63 provinces and causes a very huge economical loss. ASFV could be transmitted directly by contact pigs to pigs, or indirectly by the virus contaminated human or fomites as the vehicles, equipment… When the ASF becomes more and more complex, feed and its ingredients are also present a high possibility to carry and transmit the ASFV through out the swine production system on the world. There are many literatures about ASF clinical signs, laboratory diagnosis… However, there are always the real problems of ASF on filed, in domestic pig herds, that could not be mentioned clearly before, and that could affect the diagnostic result and in consequence makes the disease's spreading, perhaps, uncontrollably. This work presents the information concern real problems including clinical signs in domestic pig herds, as well as laboratory diagnosis, the risk of feed in ASF transmission in Vietnamese pig production system.

Materials and Methods

Serum, spleen, lung of ASF suspected pigs from the pig farms. Imported blood or meat meal from the feed mill company.

All the samples were tested ASFV with conventional PCR with primers and protocol recommended by OIE (2019). Blood and meat meal ASFV positive with conventional PCR was sent to two other Laboratories in Ho Chi Minh city - Vietnam to confirm by real-time PCR with commercial kits and PCR product was sequencing (Nam Khoa company in Ho Chi Minh city – Vietnam) and the nucleotide sequences were aligned with the ASFV sequence references on NCBI. The antibodies vs ASFV was evaluated with ELISA INGEZIM PPA COMPAC Test Kit.

Results

The observation in domestic pig herds ASFV infection in Vietnam showed the clinical signs that could not be mentioned clearly before as sub-acute, or possible chronic forms. The pigs are being ASF viraemia and high antibody ELISA vs ASFV at the same time without clinical signs of ASFV infection. In ASF acute form the pigs presented a subcutaneous hematoma that differs clearly from other diseases having similar clinical signs as Porcine reproductive and Respiratory Syndrome, Classical swine fever, Porcine Circovirus, Erysipelas…

In the case of feed ASFV contamination, OIE conventional PCR protocol for ASFV diagnosis in this study presents more sensitive in comparison with two commercial real-time PCR (table 1).

Table 1. ASFV false negative with commercial real-time PCR on dried blood samples

Dried blood samples	PCR (Hanviet Lab)	Real-time PCR (Lab 1)	Real-time PCR (Lab 2)
1	Positive	Not test	Negative
2	Positive	Negative	Negative

Conclusions and Discussion

In fact, the ASF clinical feature varies in depending on the virulence of ASFV strain… that cause difficulty in clinical diagnosis on field. Skin hemorrhagic lesion in pigs ASFV infection has been described in all literatures concern ASF, however, subcutaneous hematoma (black violet dots under the skin) observed with the ASF acute form on field have not being discussed in domestic pigs with ASFV infection. In addition, the presence of ASF viraemia and high antibody ELISA at the same time in pigs without any clinical signs of ASF in the new ASF outbreak as in Vietnam should be considered as an epidemical example to investigate more in domestic pigs. The false negative results with two commercial real-time PCR kits on the samples from suspected pigs, as well as in meat and blood meal, cause a severe problem in early ASF diagnosis and in ASFV transmission control. These false negative results showed the reliability of different commercial real-time PCR kits in ASFV diagnosis.

And what's more, ASFV DNA was detected in imported dried blood meal and meat meal causes a great risk of ASFV transmission trans-border worldwide. So, the sub-acute or chronic forms in ASF new outbreak should be more investigated, and we must pay more attention to control strictly the ASFV contamination of all feed ingredients for pigs to prevent ASF outbreaks.

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Clinical and productive performance in a production flow using three different PCV-2 vaccines from a contemporary field trial in a multisite Mexico farm

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Introduction

The use of PCV-2 vaccines to control PCV2-Associated diseases is a common practice in Mexico since vaccine authorization in Mexico, vaccine schedule in most of the cases are referred to application around weaning age (1), there are other schedules using a second dose three weeks after. Programs try to control clinical signs during all the production flow however PRRS circulation can affect vaccine performance with PMWS signs between 12 and 14 weeks age (2).

Objective

Determine the clinical and production performance from three commercial PCV2 vaccines in a multi-site system in a positive stable PRRS farm during weaning and fattening stages.

Materials and Methods

The trial was performed in a 2500 sows farm in Puebla State that produce ± 1000 piglets weekly. One production batch was divided in three sub-groups with 318 piglets each one and allocate to one of three different treatments (PCV2-vaccines); Vaccine a) Sub-unit two-dose, Vaccine b) Sub-unit one-dose and Vaccine c) Complete virus one-dose. Piglets were ear-tagged to register body weight, clinical signs and mortality, monitor pens with 25 piglets were used to measure feed intake and feed efficiency. During the weaning age recorded parameters were; Average Daily Weight gain (ADWG), Feed Efficiency (FE) and bodyweight at 10 weeks age before it´s transfer to fattening pens. For fattening stage; ADWG, sales average weight feed efficiency and mortality. Data were analyzed by SPSS V.5.0 software with normal distribution and variance uniformity assumptions (Levene test) and an ANOVA test (Tuckey) to detect significant differences ($p \leq 0.05$) between sub-groups.

Results

Tables 1 and 2 showed productive performance in weaning and fattening stages.

Table # 1. Production parameters in weaning stage.

	Vaccine a Average (Std. Error)	Vaccine b Average (Std. Error)	Vaccine c Average (Std. Error)
Mortality (%)	2.99 ^{a} (0.146)	3.79 ^{a} (0.293)	2.734 ^{a} (0.108)
Average Daily weight gain (kg)	0.45 ^{a} (0.002)	0.46 ^{a} (0.003)	0.45 ^{a} (0.004)
Feed efficiency (kg)	1.61 ^{a} (0.01)	1.61 ^{a} (0.018)	1.66 ^{a} (0.024)
Body weight adjusted at 70 days age (kg)	28.48 ^{a} (0.125)	28.70 ^{a} (0.174)	28.74 ^{a} (0.187)

Different superscripts in the same row indicate statistic differences ($p \leq 0.05$)

Table # 2 Production parameters in fattening .

	Vaccine a Average (Std. Error)	Vaccine b Average (Std. Error)	Vaccine c Average (Std. Error)
Mortality (%)	2.74 ^{a} (0.131)	3.27 ^{a} (0.254)	2.71 ^{a} (0.109)
Average Daily weight gain (Kg)	0.81 ^{a} (0.011)	0.79 ^{a} (0.008)	0.82 ^{a} (0.009)
Feed efficiency (kg)	2.58 ^{a} (0.046)	2.61 ^{a} (0.027)	2.57 ^{a} (0.033)
Body weight at sales (kg)	105.45 ^{a} (0.842)	104.79 ^{a} (0.538)	105.93 ^{a} (0.69)

Different superscripts in the same row indicate statistic differences ($p \leq 0.05$)

In this study there were an mortality increase in weaning and fattening stage in the vaccine b group although there were no significant differences detected (significance p value at weaning 0.06 and in fattening 0.09), the same group showed the highest feed efficiency in fattening (2.61) also without statistic difference

Conclusions and Discussion.

Normally PCV2 effects began to appear in grower stage where the presence of culled pigs affect performance mainly feed efficiency, in this study the problem began to detect from weaning and the feed efficiency was not affected, the mortality increase could be associated with a PRRS viremia. The same effect continue during the fattening period with an increase in feed efficiency and reduction in body weight at sales. PCV2 vaccine performance needs to be consider with PRRS circulation status in farms mainly in first parity flows. In this study there were no differences in performance for the three vaccines.

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Senecavirus A in swines attended by the Official Veterinary Service of Goiás, Brazil, in 2018 and 2019

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Introduction

Senecavirus A (SVA) affects swines causing vesicular lesions clinically similar to those found in vesicular diseases such as foot and mouth disease, vesicular stomatitis, swine vesicular disease and swine vesicular rash (1). The SVA lesions occur on the snouts and paws of animals, forming vesicles that, depending on the time of evolution, may be broken and crusting (2, 3). The responsible to find, diagnose and trigger control measures of the vesicular diseases is the Official Veterinary Service, and the direct attendance to reports of mortality or clinical signs is done by the state animal health agency (4).

Materials and Methods

Between December 2018 and December 2019, 60 cases of pigs presenting vesicular lesions were assisted by the animal health agency of Goiás. The attendance started from the notification of the veterinarians responsible for the farms, the farmers themselves and by the veterinarians of state animal health agency. After the notification, the state agricultural inspector, veterinarian, conducts a research on the farm and can substantiate the suspicion, classifying it as a probable case or a discarded case. In the first situation, samples of blood serum, epithelium, crusts and fluid from vesicles are collected. Serum and vesicular fluid samples are shipped frozen, while those of epithelium and crust are placed in Valée Liquid, chilled and sent to the Federal Laboratory of Agricultural Defense in Pedro Leopoldo - MG. Suspicious properties have the animal movement blocked until vesicular diseases are ruled out.

Results

Between December 2018 and December 2019, 60 suspected vesicular diseases in swines were attended in eight municipalities of the southwest region of Goiás. The notifications came from the sanitary veterinarians of the farms (90% of the cases) and by active surveillance of animal health agency (10% of the cases). All suspicions were considered probable cases and the sampling for laboratory diagnosis were performed. Among the 60 properties, 51 were positive for Senecavirus A, and in only eight samples it was not possible to identify the agent, however, foot and mouth disease and vesicular stomatitis were discarded. SVA were detected in Aparecida do Rio Doce, Cachoeira Alta, Jataí, Montividiu, Rio Verde e Santo Antônio da Barra (Image 1). Among the farmed in which SVA were confirmed, 63% were terminator farms, 20% were pig breeding systems, 16% certified swine breeder and 1% were full cycle farms. In ten properties, besides the positive laboratory result for SVA, an epidemiological link was found with properties already diagnosed positive for the disease.

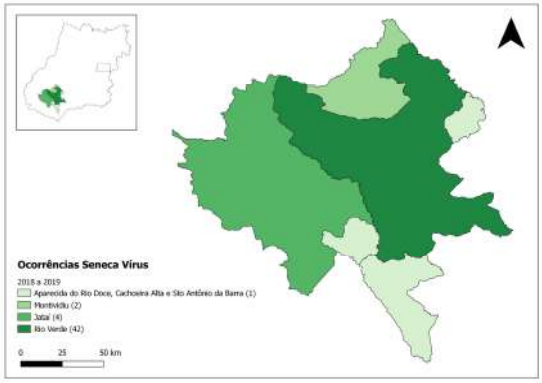

Image1: Cities distribution of *Senecavirus A* in Goiás, Brazil, between 2018 and 2019.

Conclusions and Discussion

The reports of suspected vesicular disease were mainly from farm veterinarians and it is important that the communication to official veterinary service is done quickly so that investigative measures are taken efficiently, preventing the spread of the disease and disorders during the slaughter of the animals with vesicular lesions, preventing the carcass hijacking until foot and mouth disease, vesicular stomatitis are discarded. In the assessed period, *Senecavirus A* was more frequent in finishing piglets but also affected animals in the GRSCs, mostly the younger. Lesions with vesicular characteristics are the reasons that lead to the notification of the disease, animal mortality was not frequent.

Acknowledgments

Agência Goiana de Defesa Agropecuária (Agrodefesa), Goiás, Brasil. Fundo para o desenvolvimento da pecuária em Goiás (FUNDEPEC), Goiás, Brasil.

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A serological and *qPCR* investigation into *PCV2* in intensive pig farms in China

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Introduction

Porcine circovirus associated disease (*PCVAD*) is caused by porcine circovirus type 2 (*PCV2*). PCV2 has been damaging to pig industry worldwide, and was first reported in China in the late 1990's. Since the use of PCV2 vaccines, the clinical cases of PCV2 infection have been reduced significantly in China. Our study objective was to assess the infection dynamics of PCV2 under common PCV2 vaccination programs in Chinese pig herds by evaluating PCV2-specific antibodies and viremia.

Materials and methods

From Dec. 2018 to Aug. 2019, 7,606 serum samples from 140 intensive pig farms in 26 provinces or municipalities in China, where vaccination against PCV2 with international or local brand vaccines at 2 or 3 weeks of age were implemented, were randomly collected within growing-finishing pigs at 2, 4, 6, 8, 10, 12, 16, and 20 weeks of age. All samples were tested for PCV2 antibodies by ELISA (*Synbiotics PCV2 Ab ELISA* kit). 196 samples of 10 farms (4-5 samples each) from pigs at 4,10,16,20 weeks of age were tested additionally for PCV2 viremia by qPCR. All testing was performed in the Zoetis swine disease diagnosis laboratory (Beijing). The antibody positive rate of each production stage of each pig farm was calculated, and the overall median, upper quartile and lower quartile antibody positive rates of each stage were analyzed. PCV2 copy numbers were calculated and analyzed.

Results and discussion

In general, the upper quartile, median and lower quartile of positive rates continuously decreased during 2-10 weeks of age, with the lowest positive rate at 10 weeks of age when the upper quartile, median and lower quartile were 55.95%, 20% and 0, respectively. Conversely, all the upper quartile, median and lower quartile positive rates of these farms rose during 12-20 weeks of age, peaking at 20 weeks of age when the median was 66.67% and the lower and upper quartile were 25% and 100% (Figure 1). On the other hand, the PCV2 viremia positive rate increased from 4 weeks of age (with 1 of 49 samples positive) to 20 weeks of age (with 33 of 49 samples positive); 7 of 10 farms were infected with PCV2 after 10 weeks of age, but the highest mean level of PCV2 positive samples occurred at 10 weeks of age with $10^{6.45}$ copies/ml serum (Figure 2 and Table 1).

Conclusion

This study showed that even if vaccination was commonly implemented in pig herds in China, infection occurred often and sometimes at high level of PCV2 viremia, especially between 10 and 20 weeks of age. It is necessary to take actions to monitor and improve the effectiveness of vaccination programs and reduce prevalence of PCV2 infection.

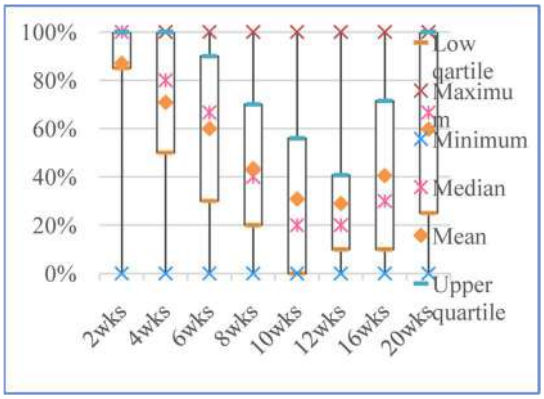

Figure 1. The low quartile, Maximum, minimum, median, mean upper quartile positive rates of 140 farms by weeks of age

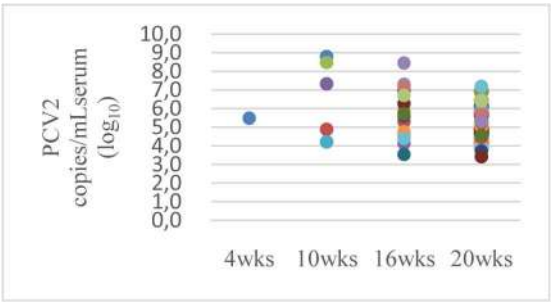

Figure 2. The number of *PCV2* copies in positive serum samples by weeks of age.

Table 1. Results of PCV2 qPCR testing of 196 samples from 4 production phases across 10 farms.

	4wks	10wks	16wks	20wks
Farms	10	10	10	10
Samples	49	49	49	49
Positive samples	1	5	17	33
Mean copies for positive samples（log $_{10}$/ml serum）	5.48	6.45	5.65	5.23
Positive farms	1	2	6	10

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Molecular epidemiology of *PRRSV* in pig herds in China based on the genetic analysis of *ORF5* from 2014 to 2018

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Introduction:
Porcine reproductive and respiratory syndrome (PRRS) continues to cause severe economic losses to the pig industry worldwide. The difficulty of controlling the disease is related to the genetic diversity of its causative agent, *PRRS* virus (*PRRSV*). The *ORF5* gene of *PRRSV* is highly variable and used to assess the variation and evolution of *PRRSV* in epidemiology survey. The objective of this study is to reveal the prevalence of diverse lineages *of PRRSV* type 2 in China from 2014 to 2018 by sequencing the *PRRSV ORF5* gene.

Materials and Methods
From Jan. 2014 to Dec. 2018, a total of 444 tissue or serum samples from 195 farms in 20 areas of China were collected and confirmed to be positive for *PRRSV ORF7* by *RT-PCR*. Sequencing of *PRRSV ORF5 gene* was conducted from these samples. The analysis for genetic evolution of the *ORF5* sequences was performed by using *MEGA6.0* software. Samples from same farms were analyzed together to determine the strain lineages prevailing. Then the prevailing rate of different lineages in farms was concluded.

Results and Discussion
All 444 *ORF5* sequences analyzed belonged to genotype 2 *PRRSV*. Based on their deduced amino acid sequences, field isolates could be classified into 4 lineages: the lineage 1, 3, 5 and 8 (Figure 1). All strains within lineage 8 could be further divided into 3 sub-lineages (8.1, 8.2 and 8.3). The strains of lineages 1, 3, 5, 8.1 and 8.3 in the samples accounted for 158, 56, 23, 11 and 196, respectively. As shown in Table 1, the single existence of lineages 1, 3, 5, 8.1 and 8.3 found in farms was 60/195, 24/195, 9/195, 3/195 and 74/195, respectively. Co-existence of two lineages 1 and 5, 1 and 8.1, 1 and 8.3, 3 and 5, 3 and 8.3, and 5 and 8.3 was found in 3/195, 1/195, 8/195, 1/195, 8/195 and 1/195 farms, respectively. Co-existence of three lineages 1, 3, 8.3, and 1, 5, 8.3 found in farms was 1/195 and 2/195. The percentage of farms with lineage 1 strain during 2017-2018 was greater compared to 2014-2016 (Table 2), but the percentage of the farms with lineage 8.3 decreased, and the number of farms with lineage 3 and 5 was maintained.

Conclusion
Lineage 1 *PRRSV* showed a clear tendency to spread widely. Controlling *HP-PRRSV* and *NADC30-like* strains should be considered in the field.

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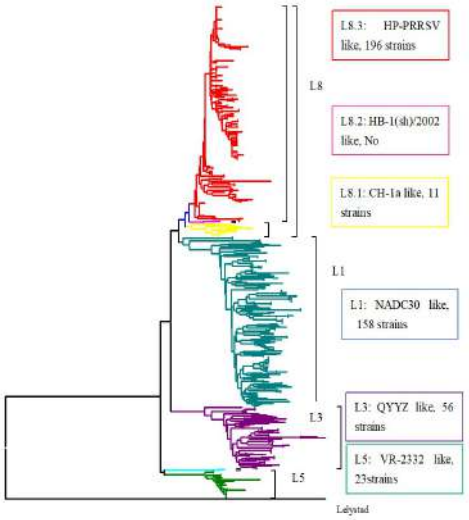

Figure 1. Phylogenetic tree based on the deduced amino acid sequences of 444 *PRRSV ORF5* genes.

Table 1 Number of pig farms with diverse lineage of *PRRSV* type 2.

PRRSV strain	Number of pig farm (percentage)
L1	60（30.77%）
L3	24（12.31%）
L5	9（4.62%）
L8.3	74（37.95%）
L8.1	3（1.54%）
L1+L5	3（1.54%）
L1+L8.3	8（4.10%）
L1+L8.1	1（0.51%）
L3+L5	1（0.51%）
L3+L8.3	8（4.10%）
L5+L8.3	1（0.51%）
L1+L3+L8.3	1（0.51%）
L1+L5+L8.3	2（1.03%）
Total	195

Table 2 Prevalence of various lineages of *PRRSV* type 2 in pig farms during two periods

	L1	L3	L5	L8.1	L8.3
2014.1~2016.1	30%	17%	8%	3%	64%
	（26/88）	（15/88）	（7/88）	（3/88）	（56/88）
2017.1~2018.1	45%	18%	8%	1%	35%
	（48/107	（19/107	（9/107	（1/107	（38/107

Detection of PRRSV-specific antibodies in fecal samples using a commercial PRRSV oral fluids ELISA – a pilot study

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Introduction

Detection of porcine reproductive and respiratory syndrome virus (PRRSV) antibody in aggregate specimens, e.g., oral fluids (OF), processing fluids, has been an active area of research in recent years because such sample provide significant advantages for surveillance at the herd level. However, testing individual animals is still necessary under specific circumstances. Serum is the historical specimen for individual animal antibody monitoring, but fecal samples (FS) are easily collected by a single person and do not require animal restrain. The presence of antibody in feces (copro-antibody) against specific pathogens has been described in humans and animals, e.g., sheep, mice, dogs, primates, etc. In swine, copro-antibody against African swine fever virus, classical swine fever virus, hepatitis E virus, and porcine epidemic diarrhea virus has been documented[1,2] but there are no reports on PRRSV copro-antibody. This pilot study describes the detection of PRRSV coproantibody in fecal samples from pigs of known PRRSV infection status.

Materials and Methods

Twelve individually-housed pigs were vaccinated with a modified live virus (Ingelvac® PRRS MLV) and sampled from -5 to 42 days post vaccination (DPV). A total of 132 serum, 572 OF, and 573 FS were collected. Serum and OF were tested using commercial specimen-specific PRRSV IgG ELISAs (IDEXX OF Ab test, IDEXX PRRS X3 Ab Test, IDEXX Laboratories, Inc). FS were tested for PRRSV IgG antibody by diluting samples (1:1) with ELISA kit diluent containing 1500 ppm chitosan (Figure 1) and then testing 200 µL of sample supernatant on the PRRSV OF Ab test ELISA using the procedure described by the manufacturer for oral fluids testing.

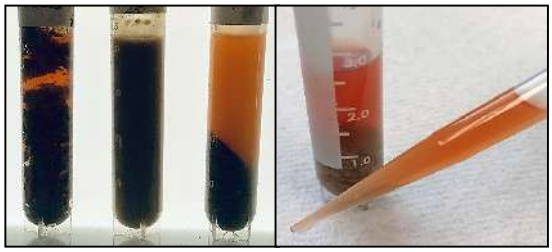

Figure 2. Fecal sample clarification process. (L) fecal sample + kit diluent (1:1), vortexed sample, clarified fecal sample. (R) sample supernatant ready for ELISA testing.

Results

The first PRRSV-antibody positive samples were detected at 8 DPV on serum ELISA and OF-ELISA using a cutoff of S/P ≥ 0.4. The first positive fecal samples appeared at 10 DPV using a cutoff of ≥ 1.0. (Figure 2). To determine and compare the diagnostic performance of ELISAs, a ROC analysis was conducted, with samples collected < 7 DPV considered true negatives and samples collected ≥ 12 DPV as true positives. The diagnostic sensitivity - specificity of both the serum and OF-ELISAs were estimated as 99% - 99% respectively, whereas the FS-ELISA were 81% - 99%.

Conclusions and Discussion

This pilot study demonstrated that PRRSV-specific antibodies are present in swine feces and that copro-antibody ontogeny mirrors serum and oral fluid PRRSV antibody kinetics (Figure 2). Future research will focus on the development of an ELISA assay specifically for fecal samples and a practical approach for collecting fecal samples to test adult animals (sows or boars) in PRRSV surveillance programs.

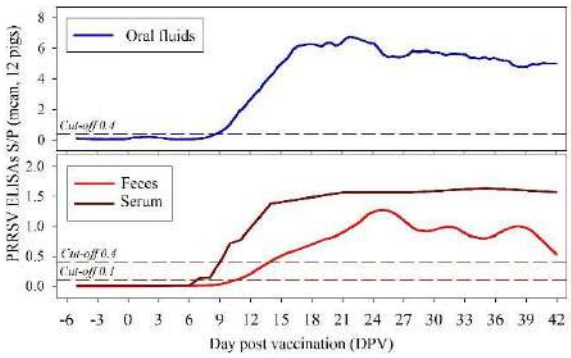

Figure 1. PRRSV antibody detection (mean S/P of 12 pigs) in serum, oral fluids, and fecal samples over time.

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The authors declare no conflicts of interest (COI) with respect to this study. Co-author JZ has consulted with IDEXX Laboratories, Inc. on areas independent of this research. Consulting has been reviewed and approved by ISU in accordance with its COI policies.

Technician effect on PRRSV OF Ab ELISA test results, a key detail.

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Introduction

Oral fluids (OF) is the most popular diagnostic specimen used in PRRSV surveillance[1]. Veterinary diagnostic laboratories usually use commercial OF ELISA kits for PRRSV-antibody detection which have demonstrated to provide satisfactory diagnostic performance[2]; however, the person who performs the ELISA test may play a role in results repeatability. This study quantify the impact of the technician on a commercial PRRS OF antibody ELISA test results.

Materials and Methods

Oral fluid samples (n = 600) from experimental pigs of known PRRSV status were tested on IDEXX PRRS OF Ab ELISA test (IDEXX Laboratories, Inc.). Samples were randomly allocated in seven predilution plates and the ELISA test performed independently by two trained technicians (T1 and T2) using the same laboratory equipment. Assay reactions were converted to sample-to-positive ratios (S/P) and the cutoff (≥ 0.4) interpreted the S/Ps as positive or negative results. Non-parametrical analyses were performed to analyze the ELISA results data (T1 vs T2) as follow:

1) ROC-Area under the curve to compare the tests diagnostic performance.
2) Wilcoxon test to compare qualitative S/P ELISAs results.
3) Cochran's Q test to compare the proportion of positive and negative PRRSV antibody results.

Results

1) No difference was detected between T1 or T2 results in terms of diagnostic performance. ROC-AUC: 0.997 (ROC-AUC pairwise comp., $p = 0.41$).
2) T1 systematically produced higher S/P results than T2 (median S/P 1.99 vs 1.91, Wilcoxon, $p < 0.5$) (Figure 1).
3) T1 produced more positives than T2 (393 vs 390). However, no significant difference was found between the proportions of positive results (65.5% vs 65%) (Cochran Q, $p = 0.18$) (Figure 1).

An investigation into the cause of the quantitative differences in results revealed that the T1 loaded the kit controls immediately after the samples to avoid samples cross-contamination, whereas the T2 loaded the positive and negative kit controls before loading the samples (as suggested by the kit insert). This difference in control incubation time affected the kit positive controls ODs (Figure 2) and, therefore, affected the sample S/Ps calculations. As consequence, differences in interpretation of T1 and T2 results as negative or positive were observed. Specifically, on those oral fluid samples with S/Ps results close to the cutoff (S/P ≥ 0.4), affecting the early detection of PRRSV-specific antibody.

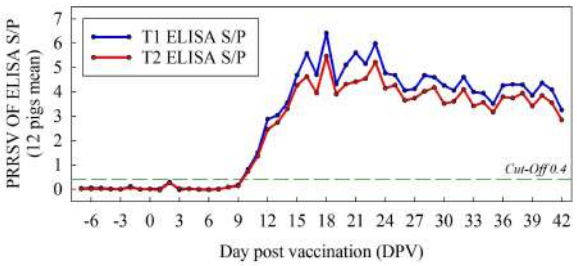

Figure 1. T1 vs T2 PRRSV OF Ab ELISA S/P results on oral fluids (n = 600) from 12 PRRSV MLV vaccinated pigs over time.

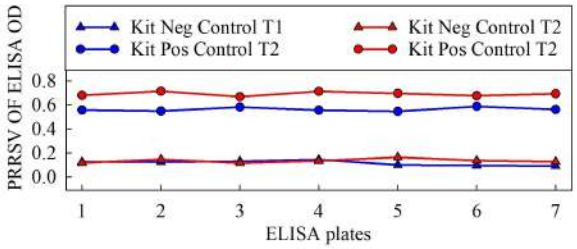

Figure 2. T1 and T2 PRRSV OF ELISA kit controls Optical Densities (OD) results.

Conclusions and Discussion

During any testing process, a variety of factors can affect the test results, e.g., sample handling, incubation time, temperature, equipment calibration, and others. Veterinary diagnostic laboratories implement a variety of standard operating procedures (SOP) to control for these factors.

This study found that even very small differences in performing a test (technician effect) could introduce a measureable impact on testing results. Although the PRRS OF ELISA Ab test is highly robust and thus the deviation from the recommended procedures did not affect significantly the binary (negative or positive) diagnostic results, it underlined the fact that test precision and test repeatability are dependent on attention to detail.

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1. Trevisan G, et al. 2019. PLoS One. 14:1–16.
2. Henao-Diaz A, et al. 2019. Res Vet Sci. 125:113–8

The authors declare no conflicts of interest (COI) with respect to this study. Co-author JZ has consulted with IDEXX Laboratories, Inc. on areas independent of this research. Consulting has been reviewed and approved by ISU in accordance with its COI policies.

Continual process improvement - the case of the PRRS oral fluid ELISA

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Introduction

PRRSV surveillance requires an easily collected specimen and accurate diagnostic tests. Oral fluid (OF) is the most common surveillance specimen in the US[1] and both RT-PCR and OF ELISA have been adapted to the matrix. Detection of PRRSV RNA provides the advantage of early detection; PRRSV antibody provides the advantages of lower cost and longer duration of detection.

In routine surveillance, unexpected positives cause disruption in the work flow on the farm and undermine client confidence in the laboratory. Hence, diagnostic specificity (DX-SP), **not** diagnostic sensitivity (DX-SE), is the major consideration.

Under experimental conditions, the DX-SE and DX-SP of the PRRS OF ELISA were estimated at ~100%[2] using the manufacturer cut-off S/P 0.4. Herein, we evaluate the DX-SP of the PRRS OF ELISA using field samples and provide alternative cutoffs (S/P 0.6, 0.8, and 1.0) for surveillance.

Materials and Methods

Two sets of OFs samples were used:

Set 1: 596 OFs of known negative and positive status.

Set 2: 1574 OFs from presumed PRRSV-naïve sites.

Set 1 samples were generated under experimental conditions and Set 2 consisted of samples submitted for routine testing at the Iowa State University Veterinary Diagnostic Laboratory. All samples were tested on the PRRS oral fluid Ab ELISA (IDEXX Laboratories Inc.) and the data analyzed using non-parametrical statistical procedures:

- DX-SE and DX-SP analyzed by receiver operating characteristic curve (ROC) analysis (Set 1);
- ELISA S/P by pig age analyzed using linear regression and Tukey's box plot (Set 2);
- Effect of alternative cut-offs on positivity rates analyzed using Cochran's Q (Sets 1, 2)

Results

Set 1: As shown in Figure 1, at a cut-off of S/P ≥ 0.4, DX-SE and DX-SP were both > 99.4%. Higher S/P cut-offs did not improve DX-SP because of the limited number of negative samples (n = 167), but likewise had minor impact on DX-SE.
Set 2: Figure 2 shows the 99th percentile S/P response by pig age or category. "Extreme S/P outliers" (n = 48; Tukey's box plot) were associated with specific categories, i.e., 9, 24, 25 weeks of age and gestation. Table 1 provides DX-SP estimates (with and without "extreme S/P outliers") at alternative cut-offs.

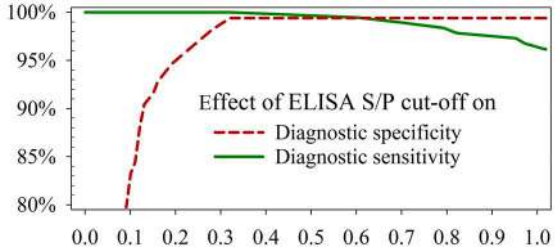

Figure 1. PRRS OF ELISA DX-SE and DX-SP (Set 1).

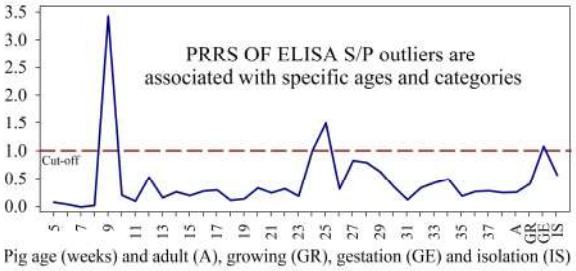

Figure 2. PRRS OF ELISA (99th percentile) (Set 2).

Table 1. PRRS OF ELISA DX-SP by cut-off (Set 2).

Set 2. Field samples of known PRRSV negative status				
	Set 2 (n = 1657 OF)		Set 2 (n = 1609 OF)[1]	
Cut-off (S/P ≥)	False Pos	DX-SP (%)	False Pos	DX-SP (%)
0.4	78[a]	95.3	30[a]	98.1
0.6	39[b]	97.6	0[b]	100
0.8	23[b,c]	98.6	0[b]	100
1.0	13[c]	99.2	0[b]	100

[1] Excludes 48 extreme S/P outliers (Tukey's box plot analysis).
[a,b,c] Different letters indicate statistical differences in the proportion of positives within the column (Cochran's Q, $p < 0.05$).

Conclusions and Discussion

Routine PRRSV surveillance demands near-perfect diagnostic specificity, even at the cost of diagnostic sensitivity. Using a cut-off of S/P ≥ 1.0 significantly reduced false positives (Table 1) and minimally affected DX-SE (Fig 1). This cut-off is recommended for the interpretation of PRRS OF ELISA results from presumed negative sites.

Notably, extreme S/P responses were associated with PRRSV-naïve pigs of specific ages and/or in gestation. Work in progress is expected to identify the cause(s) of this non-specific response.

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1. Trevisan G, et al. PLoS One. 2019;14:1–16.
2. Henao-Diaz A, et al. Res Vet Sci. 2019;125:113–8.

Authors declare no conflicts of interest (COI). JZ has consulted with IDEXX Laboratories, Inc. independent of this research. Consulting has been reviewed and approved by ISU in accordance with its COI policies.

Pseudorabies virus (PRV) antibody detection in serum and oral fluid specimens

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Introduction

Pseudorabies virus (PRV) can be eradicated from domestic swine using marker vaccines and accompanying ELISA(s) (1,3). However, PRV circulates in free-ranging feral swine populations on all continents, with documentation of occasional spillover into domestic herds (2). This on-going threat to PRV-free swine populations justifies efforts to improve PRV surveillance. Thus, the objective of this study was to evaluate the detection of PRV antibodies in swine oral fluid.

Materials and Methods

Oral fluid and serum samples were obtained from 12- to 16-week-old pigs allocated to 4 groups (10 pigs per group): 1) negative control (NC), 2) wild-type PRV inoculation (PRV 3CR Ossabaw), 3) PRV vaccination (Ingelvac® Aujeszky MLV), and 4) PRV vaccination followed by PRV inoculation (PRV 3CR Ossabaw) at 21 days post vaccination (MLV-PRV). Depending on the group, serum and oral fluid samples were collected from individual animals for up to 49 days. Serum and oral fluid samples were tested for PRV antibody using a PRV whole virus indirect IgG ELISA; oral fluid samples were tested using a modified protocol. ROC curve and repeatability analyses were performed to evaluate assay performance.

Results

PRV antibody ontogeny in serum samples and oral fluids showed a similar response pattern (Figure 1). PRV antibody was detected in serum by 7 days post vaccination (MLV-PRV and MLV) or inoculation (PRV); in oral fluid by 10 days after inoculation (MLV-PRV and PRV). A rapid and strong anamnestic response was observed in MLV-PRV pigs. In contrast, no serum or oral fluid antibodies were detected in NC group.

Conclusions and Discussion

PRV-vaccinated/-inoculated pigs generated detectable antibodies in oral fluid over time, i.e., 1) PRV-specific antibodies could be detected in oral fluid specimens and 2) the antibody ontogeny induced by wild-type PRV was comparable in oral fluid to that in serum. Research in progress will focus on the optimization of the commercial PRV indirect ELISA for oral fluids and estimation of diagnostic sensitivity/specificity and assay repeatability.

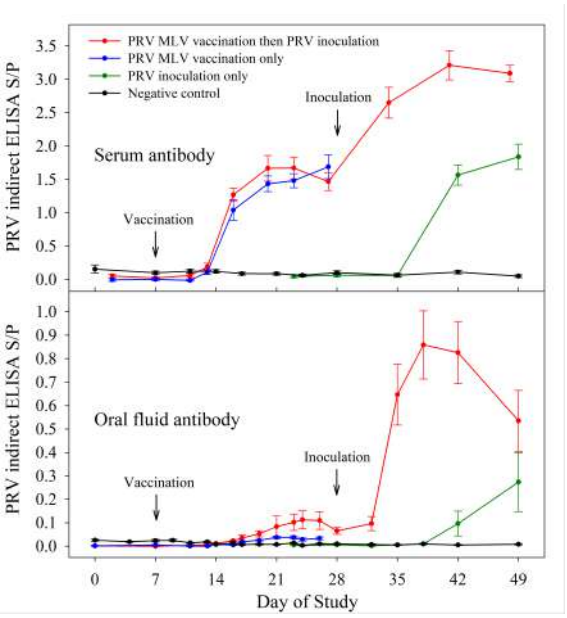

Figure 1. Serum and oral fluid PRV antibody responses ($\bar{x}$, standard error) based on ELISA testing.

Acknowledgments

PRV indirect ELISA kits were provided by IDEXX Laboratories, Inc. (Westbrook, ME). This research was funded by Swine Health Information Center, Ames, IA.

Declaration of conflicting interests

The authors declare no conflicts of interest with respect to their authorship and/or the publication of this manuscript, with the exception that J. Zimmerman serves as a consultant to IDEXX Laboratories, Inc. on areas of diagnostic medicine independent of this study.

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Phylogenetic analysis of ORF5 gene of porcine reproductive and respiratory syndrome virus detected in Peru from 2015 to 2017

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Introduction

The first evidence of porcine reproductive and respiratory syndrome virus (PRRSV) presence in Peru dates back to 1998, when antibodies were detected in 13.6% of finishing pigs (1). Later, presence of European genotype PRRSV was detected by isolation and RT-PCR in pigs with no clinical signs in Lima and Arequipa provinces of Peru (4). In 2015, in contrast to the absence of clinical signs we identified different clinical patterns in highly technified farms as abortions (10-50%), fever close to 42°C, lack of appetite and high levels of antibodies against the PRRSV. Hence, the objective of the study was the Phylogenetic analysis of ORF5 gene of PRRSV strains in farms from Lima and Arequipa in 2015 to 2017 period.

Materials and Methods

A total of 42 serum samples from well managed pig farms with clinical signs compatible with PRRS were selected from the serum bank of the Virology Section, Microbiology and Parasitology Laboratory, College of Veterinary Medicine, National University of San Marcos (Peru). The detection and genotyping was performed by real time RT-PCR using a commercial kit (Tetracore, USA). The sequencing and phylogenetic analysis was carried out by amplifying the complete ORF5 (603nt) gene of the PRRSV using our designed primers and conventional RT-PCR protocols.

Results

95.2% (40/42) of samples were positive to the American genotype of the PRRSV (NA-PRRSV). None of the samples amplified for the European genotype. The sequencing and phylogenetic analysis of 20 samples from 15 highly technified farms (Lima=12 and Arequipa=3) showed that all Peruvian strains belong to a single clade of the subgroup 2 of the NA-PRRSV and are closely related to the highly virulent PRRSV strain IA/2014/NADC34 (GenBank accession number MF326985), sharing ~99% nucleotide identity with this PRRSV strain. Nucleotide identities between the Peruvian strains characterized here varied between 97% and 99% in ORF5.

Conclusions and Discussion

Twelve of the 15 farms reported clinical manifestations compatible with PRRSV infection. Notably, the most common clinical sign was abortion, the remaining farms did not evidence any clinical sign. However, we were able to detect PRRSV. Subclinical/persistent PRRSV infection can facilitate silent spread of the virus within and between farms by carrier animals (2,3).

Additionally, all strains were grouped using the RFLP classification system as previously described (6). In the study 75% of the strains detected during these outbreaks (2015-2017) were associated with PRRSV 1-7-4 strains (5).

Our results demonstrate the origin of the Peruvian strains in a single clade belong to subgroup 2 of NA-PRRSV related to the highly virulent PRRSV strain IA/2014/NADC34.

Acknowledgments

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Isolation and phylogenetic analysis of an emerging strain of swine deltacoronavirus in Peru

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Introduction

Porcine Deltacoronavirus (PDCoV) was a new virus first found in pigs in China in 2012. The second report was in United States in February 2014 and it was confirmed in Canada in March 2014 (1). PDCoV belongs to the *Coronaviridae* family as porcine epidemic diarrhea virus (PEDV) and transmissible gastroenteritis virus (TGEV). These three coronaviruses cause acute, highly contagious enteric disease, characterized by aqueous diarrhea, vomiting and severe dehydration, which could reach to 100% mortality in under 7 days old piglets (2). In Peru, TGEV was reported only in pig farms of Lima from 2011 to 2012 period (3) while PEDV cases were reported for the first time in 2014 and to date sporadic foci are present in different farms on the coast of Peru (4).

PDCoV has been considered an exotic disease for Peru, because there was no evidence of its presence. However, in November 2019 one technified farm located outside Lima presented watery diarrhea and vomiting in gilts, suckling piglets and pregnant sows causing high morbidity and low mortality. Thus, our objective was to isolate and detect the presence of PDoCV in Peru.

Materials and Methods

We analysed 3 watery feces (pool 1) and 3 whole intestines (pool 2) from gilts, suckling piglets and pregnant sows. Both pools were processed for virus isolation and molecular detection using a specific kit multiplex real time RT-PCR for differentiation of PEDV, TGEV and PDCoV in one reaction (Tetracore, USA).

Briefly, for Isolation and RNA extraction, samples were diluted 1:5 (v/v) in phosphate buffer solution and centrifuged at 3500 RPM for 10'. Supernatants were filtered and kept frozen (-80ºC) until use. Both samples were inoculated in Vero-76 cell line in trypsine-containing medium (10µg/ml). Cytopathic effect (ECP) was observed by day 2 post inoculation (p.i). For RNA extraction, we used a commercial silica based method (Norgen, Canada). The detection and differentiation of the three coronaviruses (TGEV, PEDV, PDCoV) were run following the manufacturer's instructions (Tetarcore, USA). Next generation sequencing was performed on RNA isolated from cell culture-derived virus as previously described[5].

Results

Both samples (pool 1 and 2) presented ECP in inoculated Vero-76 cell line by day 2 p.i. Morphological changes were characterized by enlarged, rounded and densely granular cells. Viral isolations were confirmed as PDCoV through real time RT-PCR and sequencing. There was no amplification of TGEV and PEDV. In addition, positive samples showed a threshold between 9.5 and 13.39 cycles. The complete gene sequence of the isolated PDCoV was >99% identical to PDCoV identified in Minnesota, U.S. in 2014.

Conclusions and Discussion

PDCoV has been considered an exotic enteric disease to Peru, because there was no evidence of its presence until now. This abstract represents the first documented report of the PDCoV in Peru using viral isolation, real time RT-PCR and confirmed by full-length genome sequence. The high sequence identity of Peruvian PDCoV to U.S. strains warrants further epidemiological investigations concerning its introduction to Peru.

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Porcine circovirus type 3 in free-living wild boars in Brazil

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Introduction

Porcine circovirus type 3 (PCV3) was firstly identified in domestic swine in the US (4,5) and subsequently in several countries, including Brazil (1,7). PCV3 is detected in both sick (4,5) and healthy pigs (1). Domestic swine and wild boars are susceptible to infection with similar viral and bacterial pathogens. Although PCV3 has been detected in wild boars in Germany (6), Italy (1) and Spain (3), information about PCV3 circulation in wild boars worldwide is scarce. The aim of this study was to investigate PCV3 occurrence in free-living wild boars in Brazil.

Materials and Methods

Serum and/or lung samples from 70 wild boars were collected between January of 2017 and June of 2019 in four cities (Castro, *n* = 40; Carambeí, *n* = 6; Ponta Grossa, *n* = 15; and Teixeira Soares, *n* = 9) located in Paraná state, Brazil. Sampling collection was performed by officially authorized exotic wildlife controller agents, according to IN 03/2013 of IBAMA (2). Viral DNA was extracted from sera (*n* = 70) and lung tissue (*n* = 7) using DNeasy Blood & Tissue Kit (Qiagen®). PCR assays for diagnostic and sequencing were performed using specific primers for PCV3 (4). The PCR products were gel-purified using BigDye XTerminator Purification Kit (Qiagen) and nucleotide sequences were determined using an ABI3130xl Genetic Analyzer. The obtained sequences were analyzed and assembled with the Phred/Phrap/Consed software. Phylogenetic analyses were performed using Neighbor Joining (NJ) method in MEGA 6.0 software. ORF2 nucleotide (nt) sequences were compared with other ORF2/PCV3 sequences available in GenBank.

Results

PCV3 was detected in 7 out of 70 (10%) sera and in 5 out 7 lungs. PCV3 positive samples were from wild boars sampled in Castro and in Ponta Grossa, between 2017 and 2018. The three obtained sequences were grouped in PCV3c genotype cluster and the ORF2 sequences presented a 100% of similarity among them (Figure 1). The nt similarities between the PCV3 sequences presented here and those available on GenBank are 98.7-99.8% for Brazilian commercial pigs, and 98.6-99.2% for wild boar samples worldwide.

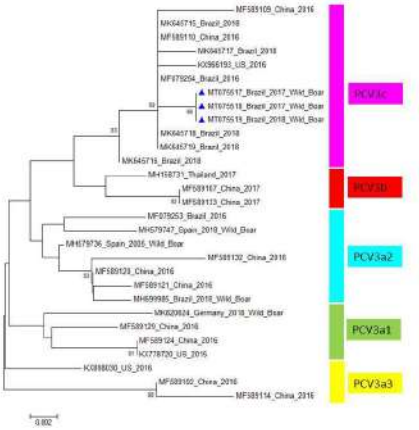

Figure 1. Phylogenetic tree of ORF2 sequences using the NJ method. The three Brazilian PCV3 sequences are labeled with a solid blue triangle.

Conclusions and Discussion

This is the first report of PCV3 in free-living wild boars in Brazil. The identity matrix revealed a high similarity between the three PCV3 sequences from this study and other sequences available in GenBank. As previouly reported, wild boars are susceptible to PCV3 infection (6) and the virus has been detected in pig herds in Brazil (7). Previous studies have suggested that occurrence of PCV3 infection in commercial pigs is higher than in wild boars (1,3) implying the role of pig management in PCV3 transmission and survival. Currently, there is no evidence that PCV3 causes disease in wild boars. However, PCV3 prevalence in wild boars should be evaluated to establish the viral dynamic in these pig population.

Acknowledgments

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Not so Bad After All: Commensal Viruses in Pigs

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Introduction

Viruses, as opposed to bacteria, are usually still primarily regarded as pathogenic, even though research shows increasingly that many viruses can be found in healthy organisms and that they indeed can be commensal or even mutualistic (beneficial) for their host. Next Generation Sequencing (NGS) facilitates the search for commensal or mutualistic viruses among the virome (entirety of viral nucleic acids) of an organism. Pigs are interesting model organisms for virome analysis, since they display a high diversity of viruses and, as omnivores, have a somewhat similar digestive tract to humans. Better knowledge about commensal enteric viruses in pigs could on one hand improve animal health regarding diarrhea, an important factor for loss in pig production, and on the other hand support research in human health.

Materials and Methods

We analysed the faecal virome and bacteriome of 50 healthy and 50 diarrhoeic piglets by NGS in order to find candidate viruses for commensalism that were positively correlated with health and commensal bacteria, and negatively correlated with pathogenic viruses and bacteria. Further quantification by (RT-)qPCR was performed to analyse differences in viral load of the candidate viruses in the two groups.

Results

Of the 27 different virus-genera found in our samples, nine were associated with health and commensal bacteria. Five of them were chosen as candidate viruses, namely Kobu-, Ungulate Bocaparvo-, stool-associated circular ssDNA virus, Adeno-associated and Porprismacovirus. Rotavirus A was negatively correlated with health and commensal bacteria. Interestingly, the healthy piglets had a significantly higher viral diversity than the diarrhoeic ones, just as older piglets compared to younger ones, regardless of their health status. The bacterial diversity behaved similarly, with significantly higher diversity indices in healthy and older animals. The (RT-)qPCRs revealed significantly higher viral loads in healthy compared to diarrhoeic piglets for Kobu-, Ungulate Bocaparvovirus 5, Adeno-associated as well as Porprismacovirus.

Conclusions and Discussion

We were able to expand the knowledge about the composition of the faecal virome of healthy and diarrhoeic piglets in Switzerland (Fig. 1), and to determine candidate viruses for commensalism in pigs may even be beneficial for the host.

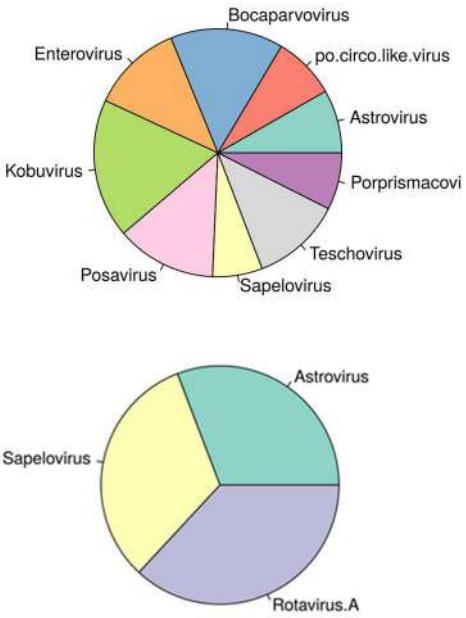

Figure 1. Medoid (representative) virome of a diarrhoeic (left) and a healthy (right) piglet

Further research will have to assess their function and biology in order to possibly prevent diarrhoeic diseases in piglets and maybe humans.

Acknowledgments

Swiss pig health association (Schweinegesundheitsdienst, SGD), Institute of Veterinary Pathology, University of Zürich.

Evaluation of the diagnostic performance of RealPCR* CSFV RNA Test, a new reverse transcriptase real-time PCR for detection of classical swine fever virus (CSFV) RNA

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Introduction

Classical swine fever (CSF) is a highly contagious, OIE-listed disease of pigs that is endemic in parts of Eastern Europe, Asia and Central and South America. Prompt laboratory confirmation in CSF-suspected cases is a key part of control and elimination strategies in CSF-free regions.

This study evaluates the performance of a new RT-PCR for the detection of CSFV.

Materials and Methods

Fifty CSFV isolates (genotypes 1.1, 1.2, 1.3, 1.4, 2.1, 2.2, 2.3 and 3.4) were used to test the inclusivity of the RealPCR CSFV RNA Test (RealPCR CSFV) [IDEXX Laboratories, Inc., Westbrook ME, USA]. Sixty CSFV-positive blood, serum and tissue (kidney, lymph-node, spleen and tonsil) samples and 80 samples (blood, serum, fecal swabs, oral swabs and tissues) from experimental infections were used to compare the performance of RealPCR CSFV and an accredited PCR [PCR-1] (1). The limit of detection (LOD) was analyzed using log10 dilutions of three CSFV-positive sera and compared to PCR-1 and a commercial PCR kit, virotype CSFV RT-PCR kit, Indical [PCR-2].

This study was performed at the Institute of Virology, University of Veterinary Medicine Hannover, Foundation, Germany.

Results and Discussion

The inclusivity study showed that RealPCR CSFV detected all the strains and genotypes tested, representing the genetic variability of CSFV.

Compared to the accredited PCR-1, RealPCR CSFV showed comparable or improved sensitivity, with lower average Ct values in all sample types, particularly in tissues (Table 1).

Table 1. Mean cycle threshold (Ct) values of IDEXX RealPCR CSFV RNA Test and an accredited PCR (1).

Sample type	Mean Ct values		
	RealPCR CSFV	PCR-1	Δ Ct
Blood	19.3	20.1	-0.8
Serum	18.1	19.0	-0.9
Spleen	16.0	18.6	-2.6
Kidney	19.7	22.3	-2.6
Lymph node	16.0	18.2	-2.2
Tonsil	16.1	17.9	-1.8

RealPCR CSFV correctly identified 80 samples (100%) from experimentally infected pigs whereas the accredited PCR-1 failed to detect one blood and two fecal swab samples containing low genome loads. At the highest PCR-positive dilution, only RealPCR CSFV was able to detect CSFV RNA, showing a LOD of 1 log10 better than the other PCRs tested (Table 2).

Table 2. Limit of detection (Ct values) of three CSFV RT-PCRs.

Dilution	Ct values		
	RealPCR CSFV	PCR-1	PCR-2
Genotype 2.1			
10^{-1}	30	33.2	29.6
10^{-2}	32.1	35.4	33
10^{-3}	36.2	-	-
10^{-4}	35.1	39.2	nt
Genotype 2.2			
10^{-1}	29.3	30.6	nt
10^{-2}	31.3	33.8	38.8
10^{-3}	33.6	-	-
10^{-4}	-	-	-
Genotype 2.3			
10^{-1}	30.2	31.6	nt
10^{-2}	32.8	34.6	34
10^{-3}	36.2	-	-
10^{-4}	-	-	-

nt, not tested.

Conclusions

In summary, this study shows that the RealPCR CSFV RNA Test is highly inclusive, with better diagnostic sensitivity and LOD than the other PCR tests evaluated in the study.

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PRRSV detection using population samples over time in different age groups in breeding herds attempting to consistently wean PRRSV-negative piglets

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Introduction

One of the major challenges of successfully eliminating PRRSV from breeding herds is determining when the within-herd PRRSV transmission has ceased which leads to consistently producing PRRSV-negative pigs at weaning. Processing fluids (PF)(1) and family oral fluids (FOF)(2) have been described as 'population samples' that can be used for monitoring of swine populations in breeding herds by sampling pigs at 3-5 days of age and due-to-wean pigs, respectively. However, there is still limited data on the dynamics of PRRSV detection over time by these methods in herds undergoing virus elimination.

The objective of this study was to describe the dynamics of PRRSV RNA detection by rRT-PCR in suckling piglets over time in herds undergoing PRRSV elimination through the use of PF and FOF.

Materials and Methods

Seven conveniently selected breeding herds attempting PRRSV elimination were monitored weekly through PF and FOF. The PCR results of each specimen were compared and described within herd over time (weeks) and farrowing rooms, and described in an aggregated format.

Results

Among all farms there was intermittent weekly detection of PRRSV RNA using PF or FOF in 15 and 7 occasions, respectively. Within the same week, positive and negative results were obtained between rooms 22 times with PF and 12 times with FOF. One farm achieved 11 weeks of consecutive negative PF results; however, FOF testing piglets at weaning detected PRRSV twice during that same period.

Overall PF and FOF results agreed in 73% of the occasions (table 1). FOF detected particular weeks as positive 9.5% of the time whereas PF tested negative for those specific weeks. Also, in 17.5% of the times that PF yielded a positive result for a specific week FOF failed to detect the same week as positive. However, when only considering matching results where the same rooms were tested by both PF and FOF, both techniques agreed for a given week 80.7% of the times. Weeks where PF classified a week as positive but FOF as negative occurred in 8.8% of the occasions, FOF classified a week as positive and PF as negative occurred 10.5% of the time (table 2).

Table 1. Overall agreement between PF and FOF on the classification of matching weeks tested by PCR for PRRSV RNA detection including all matching weeks (all herds combined).

		FOF		
		+	-	Overall agreement
PF	+	12 (19.0%)	11 (17.5%)	
	-	6 (9.5%)	34 (54.0%)	
	Total	18 (28.6%)	45 (71.4%)	73.0%

Table 2. Overall agreement between PF and FOF on the classification of matching rooms tested by PCR for PRRSV RNA detection (all herds combined).

		FOF		
		+	-	Overall agreement
PF	+	12 (21.1%)	5 (8.8%)	
	-	6 (10.5%)	34 (59.6%)	
	Total	18 (31.6%)	39 (68.4%)	80.7%

Conclusions and Discussion

The results of this study provide insights for the design of monitoring programs for breeding herds attempting PRRSV elimination. More specifically, it supports that the combination of PF (3-5 days old) and FOF (due-to-wean litters) provides an increased probability to detect the virus in the suckling pig population. The study also provides evidence of the intermittent nature of PRRSV RNA detection in different weeks and by room within the same week with PF and FOF, which demonstrates a crucial importance of continuously monitoring on a weekly basis, by sampling as many rooms as possible in an attempt to minimize misclassification of farm based on the test results of pigs in a single room Moreover, this study provided evidence that a period of more than 11 weeks of consecutive negative results with PF and FOF is necessary to establish a herd as stable for PRRSV. PF-based monitoring over time appears to be a great screening process. FOF is a great addition to the monitoring program when PCR results of PF samples start to consistently become negative for at least 8 consecutive weeks.

Acknowledgments

National Pork Board, USA for funding.

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The NC229 Multi-Station Research Consortium on Emerging Viral Diseases of Swine: Solving Stakeholder Problems through Innovative Science and Research

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Introduction

The NC229 research consortium was created in 1999 in response to the emergence of porcine reproductive and respiratory syndrome virus (PRRSV). The project follows the format for Multistate Agricultural Research driven through the US' State Agricultural Experiment Stations (SAES), wherein stakeholder-driven needs to combat swine infectious diseases are identified and scientific solutions pursued by combining funds from federal, state, commodity groups, and the animal health industry. Since its beginning, NC229 followed the model for multistate research derived from the Hatch Act of 1887 (federal legislation that gave organic form and functional structure to agricultural teaching and research in the United States). The Hatch Act defined Multistate Research Funds (MRF). The specific purpose of Hatch MRFs is to conduct research by institutions within a state in conjunction with institutions in other states to solve mutual problems that no one state could address by itself. Thus multistate research allows SAES to interdependently collaborate in projects that two or more states share as a priority. Currently, the mission of the NC229 multistate research program is to enable research on high-priority topics e.g. **PRRSV and other major emerging viral diseases of swine**, among SAES of the North Central Region of the Association of Experimental Station Directors[1] in partnership with the Agricultural Research Service and the National Institute of Food and Agriculture (NIFA) of the U.S. Department of Agriculture, other research institutions and agencies, and the Cooperative Extension Service.

Strategy followed by the NC229 consortium throughout 20 years of existence

It was primarily the "consortium-like" mentality that led the NC229 group to the active collaborative preparation of major external competitive research grants offered by USDA NIFA. NC229 researchers became the driving force for the preparation and successful award of such initial USDA-CSREES Coordinated Agricultural Project (PRRS CAP I) in 2004-2008, which, following its renewal (USDA-NIFA's PRRS CAPII) in 2009-2014, led to the overall record achievement of almost 10 million dollars available for research, extension, and education in PRRS and related diseases. The CAP funding also enabled the group to leverage swine industry funding of more than 34 million dollars, on PRRS during the last 20 years. Other significant offshoots from the funding obtained from CAP II included the PHGC (PRRS Host Genomic Consortium)[1], which, thanks to additional funding received from diverse agencies and sources remains a solid foundation for discoveries on swine genetic resistance since 2007.

Some chief outcomes of NC229 along two decades of existence

The impact of the NC229 project is primarily reflected in the numerous scientific refereed publications and successful grant submissions produced by the group. Other tangible examples of creative outcomes by the NC229 include patents and intellectual property claims. Table 1(shown on the poster) provides a few examples of patents representing inventions produced by NC229 researchers that are being implemented, marketed, or explored by industry. A major outcome of the project was also in the area of outreach. The NC229 offered the appropriate forum and the foundation for the organization of an Annual PRRS Scientific Symposium that, while initiated first at a national level, grew to become a yearly event of major international scope : ***The North American PRRSV (& Other Emerging viruses) Symposium***.

Conclusions and plans for the future: 2019-2024 and beyond

Table 2 of the poster summarizes the milestones proposed by the NC229 consortium for the current quinquennial period (2019-2024) involving at least 8 different emerging viruses of swine. African Swine Fever Virus (ASFV) retains a special focus with the goal of harnessing group's expertise in promoting preparedness for the global control of ASFV.

Acknowledgments

We dedicate this work to the memory of Dr Michael Murtaugh (1951-2018, co-author of this original manuscript) and Dr. Robert Morrison (1953-2017), two invaluable participants of NC229, forever admired never forgotten.

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Porcine Epidemic Diarrhea: producing negative piglets after an acute outbreak on a shortened schedule

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Introduction

A 5000-sow farrow-to-wean facility became infected with porcine epidemic diarrhea (PED) causing severe piglet losses. To eliminate the disease, an aggressive program of exposure, cleaning, disinfection, heightened biosecurity and removal of potentially infected piglets was undertaken. Twenty-three days after PED was diagnosed at that site, piglets were born and raised without PED infection.

Materials and Methods

The 5000-sow farrow-to-wean herd submitted piglet fecal samples confirmed PED positive by PCR on 29Nov.17. These results triggered a rapid and concurrent series of events aimed at reducing long-term losses in the production flow. Piglets >10 days of age were weaned into an off-site facility. All piglets <10 days of age were euthanized to reduce viral replication and generate an inoculum. Three days of exposure by direct contact and feedback consisting of feces and intestinal contents began 1Dec.17. Under veterinary supervision, sows due to farrow within the next 17 days were systematically aborted using prostaglandin (Lutalyse, Zoetis). For those 17 days, any viable piglet born was immediately euthanized to minimize environmental viral contamination.

Farrowing rooms were emptied sequentially, based on order of delivery dates. Each farrowing room was aggressively washed using hot water and detergent. The farrowing rooms were carefully examined for any remaining organic material and then re-washed. Special attention was given to heat lamps, over-head feed lines, feed boxes and ventilation equipment. Disinfectant (Synergize, Neogen) was applied by foaming process to ensure adequate/complete coverage. It was not possible to remove animals from the gestation stalls, but the area behind each stall was cleaned/washed. Equipment and appliances were carefully cleaned in the office, kitchen, shop areas and hallways. Any questionable material was removed from the site. Any damaged equipment that could harbor organic matter or virus was eliminated. Clothing and footwear received special attention. When animal movements resumed, all hallways used were carefully washed and disinfected after each movement. Manure pits were emptied to the lowest possible level.

When farrowings resumed, personnel working with the suspected PED negative animals utilized a double bench system – following the usual shower-in process, workers would cross the first bench when leaving a common area, enter a "gray" zone and cross a second bench before entering the clean zone. Clothing was segregated in each zone.

Strict McRebel was applied when the first suspected PED negative piglets were born. For the first turn of all farrowing rooms, there was NO transfer of piglets from any litters <15 pigs/litter and NO movement between litters after 24 hours of age. Adequate colostral intake was emphasized. Personnel were not allowed to enter farrowing crates. Workers were required to change gloves, needles and scalpels between litters. Multiple sets of processing tools were used – tools not being used would reside in a disinfectant bath before being used for the next litter.

Results

The first litters suspected to be PED negative were born 22Dec.17, 23 days after the initial laboratory diagnosis. Piglets were tested weekly to confirm negative status. Each week, 60 samples were taken, one rectal swab from the smallest pig in each of 60 litters. PED PCR results from those 60 samples pooled by five were consistently favorable/negative. Clinical evidence of PED in the nursery was absent. The sow herd and downstream flow continue to produce PED-negative animal more than two years later.

Conclusions and Discussion

PED-negative piglets can be produced from a farrow-wean facility in as few as 23 days after a confirmed diagnosis of an acute outbreak. Strict adherence to protocol details (exposure, sanitation, disinfection, animal identity, personnel segregation, people movement and clinical signs) were integral to success. Clear and detailed training was essential to proper implementation.

These results do not suggest PED virus no longer existed in the facility after such a short time; rather, the strength of maternal immunity was greater than the level of exposure in the environment. Nonetheless, the absence of clinical signs and negative diagnostic results indicate the piglets survived without clinical disease. Experience indicates with time, the virus will become absent due to the lack of a susceptible host.

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Assessment of the farm environment to achieve a better control of PCV2 infection

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Introduction

Vaccination against PCV2 has proved to be effective for controlling viremia and clinical signs. However, its use has not managed to eradicate the infection in farms, which suggests that other elements may be involved in the persistence of the virus. Therefore, we performed this work to identify the farm areas where PCV2 can accumulate and persist.

Materials and Methods

For that, we used swabs to take environmental samples from four farrow-to-weaning farms which had been vaccinating against PCV2 for years. These samples, included surfaces of different farm areas, including pig areas and other areas without animal contact, staff and utensils, which were analysed by qPCR.

Results

PCV2 DNA was detected in the environment of all these farms. Overall, the offices seem to be the most contaminated areas (mean of $1.72x10^6$ PCV2 copies/swab), although viral DNA was also detected in samples from the warehouses of two farms (mean of $2,02x10^4$ PCV2 copies/swab). Likewise, staff clothes or working footwear were contaminated by PCV2 in all the farms. Samples from the perimeter were also positive in two of the farms (main entrance of the farm and loading docks, with a mean of $4.91x10^2$ and $8.77x10^2$ PCV2 copies/swab respectively). In contrast, the areas that host pigs were contaminated in just one farm. These positive samples were taken in the floor of the gestation pens ($2.63x10^3$ PCV2 copies/swab) and in the corridors of the gestation and farrowing areas ($1.74x10^5$ and $2.45x10^2$ PCV2 copies/swab respectively). All the samples from the weaning area were negative.

Conclusions and Discussion

These results suggest that the presence of the virus in elements of the warehouses and offices may be one of the reasons why the virus could not be eradicated in swine farms. Moreover, the detection of PCV2 in working clothes and samples from different areas suggests that environmental sampling could be used for locating infected subpopulations and/or areas through which the infection could be reintroduced.

Acknowledgments

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Use of BioPortal in a PRRSV outbreak in a Spanish farm

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Introduction

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is one of the leading swine pathogens[1]. The availability of sequence data from PRRSV from widespread geographic territories has enabled a better understanding of the fight against PRRS.

The sequences do not provide any information unless we compare then within a farm, a company or at a region level. During the last 9 years, Boehringer Ingelheim Vetmedica, Inc. (BIVI) in collaboration with UC Davis -and other institutions such as Iowa State University- have improved the program Disease Bioportal software to the particularities of PRRSV. BioPortal is a web-based system that can manage real time or near-to-real time a huge amount of data. Tools are available for spatio-temporal display, graphics, and phylogenetic analysis of the data.

New introduction of isolates and the evolving dynamics of the virus can be monitored by the consistent sequence of the diagnosis. Thus, the aim of this work was to monitor the genetic changes at a farm level by using Bioportal.

Materials and Methods

The study was conducted in a 1200 sows farrow-to- wean farm located in the north-east of Spain. The farm was under a PRRS 5 step process control since August 2015. Since then, the farm has been implementing 3 sow mass vaccination per year and the piglets have been receiving a dose of Ingelvac PRRSFLEX EU before weaning. Thirty piglets were bled at weaning every two months. The constant monitoring program has allowed detecting 2 different strains since its beginning. During 2016 and 2017, we detected a resident strain (strain A), and since July 2019 we were able to detect a new strain (strain FAST). Bioportal software (http://bioportal.ucdavis.edu/) was used to understand if there was any issue of internal or external biosecurity. We assumed that sequences are different when the heterology within the 606 nucleotides of the ORF5 is higher than 3%. (Figure 1)

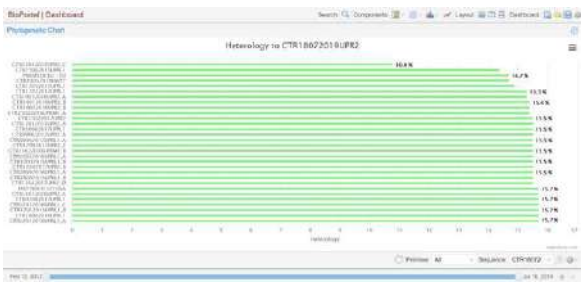

Figure 1. Simulation and phylogenetic chart, representing the sequences obtained in the farm since 2015.

In order to evaluate the impact of the introduction of a new strain in to the system several KPI were analyzed. The data were extracted from the PigCHAMP program. Two periods of time were analyzed, period before the introduction of the new strain FAST (July'18-June'19) and period after (July'19-Sep'19).

Results

Analyzing this information with the veterinarian in charge of the farm, we were able to find out that strain FAST was highly prevalent in that area since 2016, specifically it has been detected in 72 different farms of 18 different companies.

When comparing the productive results before and after the detection of the strain FAST, no differences have been detected. In this case the introduction of a new strain into the system has only affected the productive parameters in weaned piglets. The main KPI remain stable in sows. (Table 1).

Table 1. Comparison of the KPI average for the different periods.

	July'18-June'19	July'19-Sept'19
%fertility	83.6	89.3
%abortions	2.12	0.91
Total born	14.8	14.9
Live born	13.2	13.4
total death (%total born)	7.2	7.2
Mummies (%total born)	3.4	2.9
%losses 6-18kg	4%	7%

Conclusions and Discussion

Bioportal was a crucial tool to easily track a newly introduced PRRSv sequence in a farm by making comparisons within a huge sequence data base. The main conclusion is that external biosecurity is a very important risk factor for PRRSv dissemination through different areas and production systems. Special attention must be given to external biosecurity in high-density areas.

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Evaluation of two vaccination schemes against Swine Influenza Virus in fattening pigs in a commercial farm in Mexico

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Introduction

Swine influenza caused by influenza A virus (IAV-S) is an acute respiratory disease in the swine industry. IAV-S is one of the three main diseases that affect pig in all stages of production, which causes economic losses of $10.00 per animal (1), reaching up to $18.00 when associated with respiratory co-infections (2). The prevention of IAV-S could reduce the impact of respiratory co-infections and improve production parameters (3). Inactivated commercial vaccines are available; however, they had limitations to protect against Influenza virus. Recently, a live attenuated influenza vaccine (LAIV) had been develop, with a route of administration intranasal to piglets at day 3 of age. The objective of this study was to evaluate specific productive traits of pigs from weaning to finishing with two vaccination schemes against type A Influenza virus.

Materials and Methods

A prospective cohort study where carried out from September 2018 to April 2019 in a commercial farm, located at the western side of Mexico, with 4000 sows positive to Influenza virus. The farm had two sites I with independent flow from weaning (3 weeks) to finishing. Two groups of 900 pigs (180 piglets measured per week for five consecutive production weeks) where compared. The animals where individually identified with ear tags. In Group I of pigs, we use intranasal route at 3 days of age (1 mL) with a live attenuated influenza vaccine (LAIV), Ingelvac Provenza®. For the Group II of pigs, a intramuscularly vaccine was use (2 mL) at 21 and 42 days of age with an autogenous Influenza virus (H1N1/ H3N2). The vaccination program for both groups was similar: *Salmonella choleraesuis* and *Lawsonia intracellularis* (Day 7), and PRRSV MLV-*Mycoplasma hyopneumoniae*-PCV2 (Day 21). Mortality, body weight and average daily gain (ADG) data was record from weaning to finishing. The data was analyze using the statistical program (SPSS® Statistics), where the means of body weight, ADG and age at sale were compared using general linear model procedure. Mortality rate was analyze through Chi square test. Similarly, the rate of return on investment and the benefit-cost ratio were estimate using the BECAL tool (Boehringer Ingelheim Economic Calculator).

Results

The results show that there was a significant statistical difference between the vaccines being compare.

A better performance was obtained for mortality rate (Table 1), market weight and ADG (Table 2) in the pigs vaccinated with the live attenuated influenza vaccine (LAIV). These results are similar to those reported by Dykhuis et al (4) and Morgan et al (5). Those authors observed a reduction in mortality and an improvement in the ADG in pigs that were vaccinate with LAIV. Both the return on investment and the benefit-cost ratios (13.1 to 1 and 14.1 to 1, respectively) improved when the live attenuated influenza vaccine was used.

Table 1. Comparison of the mortality rate (%) in pigs under two vaccination schemes against Influenza A virus

	Vaccine group		
Phase	**Autogenous**	**LAIV**	***P*-value**
Nursery	5.89	2.95	
Growth	6.50 [a]	2.91 [b]	0.003
Wean to Finish	12.39 [a]	5.36 [b]	0.020

[a,b] Mortality rate (%) with different literal for type of vaccine were statistically significant (P<0.05)

Table 2. Comparison of productive traits in pigs under two vaccination schemes against Influenza A virus

	Vaccine group		
Traits	**Autogenous**	**LAIV**	***P*-value**
Number of pigs	900	900	
Initial body weight, kg	5.6 [a]	6.1 [b]	0.012
Final body weight, kg	99.3 [a]	112.5 [b]	0.048
Age at sale, d	180	178	
Nursery ADG, kg	0.310 [a]	0.405 [b]	0.018
Growth ADG, kg	0.737	0.821	
Wean to finish ADG, kg	0.583 [a]	0.678 [b]	0.045

[a,b] Means with different literal for type of vaccine were statistically significant (P<0.05); ADG=Average daily gain

Conclusions and Discussion

Under the conditions of this study, the average daily gain (ADG) was better for the pigs vaccinated with LAIV than the pigs vaccinated with the autogenous vaccine. This result impact directly on the productive performance, obtaining an excellent return on investment (ROI) and benefit-cost ratio (BCR).

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Evaluation of productive performance of pigs following the implementation of a vaccination program with 3FLEX® (PRSS MLV/PCV2/ *Mycoplasma hyopneumoniae*) in a fattening commercial farm in Mexico

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Introduction

Porcine reproductive and respiratory syndrome is consider one of the most devastating swine diseases affecting commercial swine production worldwide (1). PRRSV causes respiratory disease in the nursery and finishing phases, as well as reproductive failure in sows and boars (2). PRRSV infected pigs present a low performance and a high susceptibility of co-infections with secondary bacteria and other viral agents (3). The objective of this study was to evaluate specific productive traits of pigs from weaning to finishing following a vaccination scheme against with 3FLEX® (PRRS MLV/PCV2/ *Mycoplasma hyopneumoniae*).

Materials and Methods

A prospective study was carried out from April 2017 to May 2018 in a commercial farm, located at the West of Mexico. The farm had 500 sows, where the sows and piglets were PRRSV negative, and pigs in the fattening stage were positive. The farm had one site I with exclusive flow from weaning (3 weeks) to finishing. The study included 10,400 pigs (5,200 pigs per group), measured every week for twenty-six consecutive production weeks. In one group of pigs, a bivalent vaccine (PCV2+*Mycoplasma hyopneumoniae*), FLEXcombo® (Boehringer Ingelheim) applied intramuscularly route at 21 days of age (2 mL), was used. In the second group, a trivalent vaccine (PRRS MLV + PCV2 + *Mycoplasma hyopneumoniae*), 3FLEX® (Boehringer Ingelheim) was apllied intramuscularly route (2 mL), at 35 days of age. Mortality, body weight, average daily gain (ADG) and feed conversion ratio (FCR) data was register from weaning to finishing. The data was analyze using the statistical program (SPSS® Statistics), where the means of body weight, ADG, FCR and age at sale were compared using general linear model procedure. Mortality rate was analyze through Chi square test. Similarly, the rate of return on investment and the benefit cost-ratio were estimate using the BECAL tool (Boehringer Ingelheim Economic Calculator).

Results

There were significant statistical differences between the vaccines being compare. A better performance was obtain for mortality rate (Table 1), market body weight, ADG and FCR (Table 2) for the pigs vaccinated with 3FLEX®. Similar results were reported by Chae et al (4). Those authors observed a reduction in mortality and improvement in the ADG in pigs that were vaccinate with 3FLEX®.

Both the return on investment and the benefit cost ratios (15.1 to 1 and 16.1 to 1, respectively) improved when 3FLEX® was used.

Table 1. Comparison of the mortality rate (%) in pigs under two vaccination schemes

	Group		
Phase	PCV2/Mh	PRRS MLV/PCV2/ Mh	*P*-value
Nursery	1.56	1.58	
Growth	5.42 [a]	2.26 [b]	< 0.001
Wean to Finish	7.11 [a]	3.80 [b]	< 0.001

[a,b] Mortality rate (%) with different literal for type of vaccine were statistically significant (P<0.05)
PRRS MLV = PRRS Modified Live Virus; PCV2 = Porcine Circovirus Type 2; Mh = Mycoplasma hyopneumoniae)

Table 2. Comparison of productive traits in pigs under two vaccination schemes

	Group		
Traits	PCV2/Mh	PRRS MLV/PCV2/ Mh	*P*-value
Number of pigs	5,200	5,200	
Initial body weight, kg	7.3	7.3	>0.05
Final body weight, kg	114.6 [a]	121.9 [b]	0.011
Weight at 70 d, kg	30.30	30.91	>0.05
Age at sale, d	174.6	175.3	>0.05
Nursery ADG, kg	0.514	0.509	>0.05
Finishing ADG, kg	0.827 [a]	0.906 [b]	0.002
Wean to finish ADG, kg	0.722 [a]	0.761 [b]	0.014
FCR, kg	2.62 [a]	2.38 [b]	0.001

[a,b] Means with different literal for type of vaccine were statistically significant (P<0.05)
(ADG = Average daily gain; FCR = Feed Convertion Ratio; PRRS MLV = PRRS Modified Live Virus; PCV2 = Porcine Circovirus Type 2; Mh = *Mycoplasma hyopneumoniae*)

Conclusions and Discussion

Under the conditions of this study, it was observed that the average daily gain and the feed conversion ratio were better for the pigs vaccinated with 3FLEX® than the pigs without 3FLEX® vaccine. The vaccination of pigs had a direct impact on the productive performance, and on the benefit-cost ratio and the return on investment of the farm.

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Fostera® PRRS vaccination decreased mortality rate related to the concomitant infections of PRRSV and *Actinobacillus pleuropneumoniae* in Japanese swine farm

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Introduction

The control of Porcine Respiratory Disease Complex (PRDC) is a major theme in Japan as well as other countries, and porcine reproductive and respiratory syndrome virus (PRRSv) and *Actinobacillus pleuropneumoniae* (APP) infection is often occurred in finisher stage in Japan [1]. Fostera® PRRS (Zoetis Inc.), a PRRS modified live-virus vaccine, was approved for piglets at 1-day-old and older. Fostera® PRRS has already been launched in several countries in North America and Asia reporting that it has contributed to PRRS control [2]. The objective of this study was to evaluate the efficacy of Fostera® PRRS under field conditions in a farm facing PRDC where mortality was primarily related to PRRSV infection with concomitant infection of *Actinobacillus pleuropneumoniae*.

Materials and Methods

This field study was conducted in a commercial farrow to finish farm with 2,000 sows.. Pigs are weaned weekly, and housed in continuous flow site. This farm has had issues with high mortality, about 10% at finisher stage due to PRRSv and APP infection. Clear symptoms of PRRS were not observed, but PRRSv infection was recognized by PCR and serological tests at finisher stage. APP vaccine was applied to pigs at 7 and 10 weeks of age to decrease mortality, but results were not satisfactory. So, the farm decided to administrate Fostera® PRRS in pigs at 7 weeks of age instead of the APP vaccine to control PRRS from November 2018.

Blood samples were collected from specific 12 heads at 4, 7, 12, 16 and 19 weeks of age. All samples were tested for PRRS ELISA, PRRS RT-PCR. When PRRS RT-PCR was positive, the sample was tested for PRRS ORF5 sequence analysis.

The average days of age to slaughter and mortality of finisher stage was calculated during the study, and compared to past data from the farm.

Results

APP infection was still observed because the antibody titters of APP increased in the finisher stage. However, mortality rate in the stage decreased significantly ($p<0.05$) compared to the past data (Table 1). The average days of age to slaughter was not significantly different.

Table 1. Comparison of Mortality and average days of age to slaughter between the testing group and the past data.

	Number of dead over the total pigs (n)	Mortality at finisher stage (%)	Average days of age to slaughter
Past data over 3 months	461/4,311	10.7[a]	197.0
Present study	95/1,462	6.5[b]	196.8

ab, : P<0.05

Wild-type PRRS virus was detected before administration of Fostera® PRRS. Homology based on ORF5 between this wild type and Fostera® PRRS was 89.5 - 90.7%. But after administration of Fostera® PRRS, only vaccine type PRRS virus was detected (Table 2).

Table 2. Transition of PRRSv detection by ORF5 sequence analysis.

	Detection of PRRSv	
	Wild type	Vaccine type
Past data	Detected	-
Present study	ND	Detected

Conclusions and Discussion

Fostera® PRRS was effective in reducing mortality facing heterologous PRRS wild type challenge in this farm in Japan as reported in other countries [3]. Administration of Fostera® PRRS instead of APP vaccine reduced mortality in the finisher stage suffering from both, PRRSv and APP pathogens. This reduction of mortality suggests PRRSv as primary pathogen in the PRDC , highlighting the importance of controlling this virus with Fostera® PRRS to reduce the negative impact in farms facing PRDC where PRRSv and APP are involved.

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Lower intensity of tight junctions in maternal fetal interface of PRRSV2 infected fetuses

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Introduction

Existing PRRS control strategies are not completely effective and alternative approaches are needed to prevent the disease, for instance, by focusing on understanding how the fetuses are infected and then providing a foundation for elucidating a biomarker of PRRSV2 resilience. In the diffuse epitheliochorial porcine placenta, the maternal and fetal epithelium adhere to each other, resulting in interdigitations of both tissues, known as the maternal-fetal interface (MFI) (1). The MFI was suggested to be the first barrier to fetal infection and plays a role in transplacental PRRSV pathogenesis, which the mechanism is not entirely understood (2). Although intrauterine growth restricted (IUGR) fetuses appear more resilient to transplacental PRRSV infection than normal fetuses (3), the exact mechanisms are also not fully understood. Tight junction proteins (TJ) create selective barriers in the paracellular space between cells (4,5) and can be defective in some diseases, resulting in reduced or increased paracellular transport of solutes and increased permeability to large molecules (6). This is a possible mechanism of transplacental PRRSV infection. Therefore, the objective of this research is to assess TJ proteins within the MFI and search for potential alterations in integrity that affect the movement of nutrients and PRRSV across the placental barrier between different susceptibility groups.

Materials and Methods

Twenty-four paraffin embedded samples of maternal-fetal interface (MFI) from 6 non-infected (CON) and 18 PRRSV2 infected fetuses (divided equally by group; IUGR, non-IUGR, meconium stained (MEC)) were randomly selected. Five-micrometer thick tissue sections were immunostained for claudin 1 (CLDN1), claudin 4 (CLDN4) and zonula occludins (ZO1). Microscopic staining intensity was scored in 10 regions of the MFI (maternal and fetal endothelial cells (MECL/FECL), villus base maternal and fetal epithelium (VBME/VBFE), villus tip maternal and fetal epithelium (VTME/VTFE), maternal and fetal areola (MAE/FAE), glandular epithelium (GE) and amnion (AMN)) using a semi-subjective scoring system (0-absent, 1-mild, 2-moderate, 3-abundant), Image J software, and a series of custom macro functions.

Results

Differences were observed for CLDN1, CLDN4 and ZO1 (Table 1). IUGR and MEC fetuses had a lower expression of CLDN1 than control fetuses in VTFE, VBFE, MECL and FECL. Non-IUGR had lower expression of CLDN1 than control fetuses in VBFE, FECL and MECL. IUGR fetuses had lower expression of CLDN4 than control and MEC fetuses in MECL. IUGR and MEC fetuses had a lower expression of ZO1 than control fetuses in VBME, VBFE, MECL, GE and AMN. Non-IUGR had lower expression than control fetuses in AMN, VBME and VBFE.

Table 1. Median and interquartile range for CLDN1 and ZO1 in MFI regions.

	Control	CLDN1 IUGR	non-IUGR	MEC
VTFE	$3(0)^{a}$	$2.3(0.5)^{b}$	$2.3(1)^{ab}$	$1.8(1)^{b}$
VBFE	$2.8(0.5)^{a}$	$2(0)^{b}$	$1.8(1)^{b}$	$1.5(0.5)^{b}$
MECL	$1(0)^{a}$	$0(0)^{b}$	$0(0)^{b}$	$0(0)^{b}$
FECL	$1(0)^{a}$	$0(1)^{b}$	$0(1)^{b}$	$0(0)^{b}$
ZO1				
VBME	$3(0)^{a}$	$2(0.5)^{b}$	$2.5(0.5)^{b}$	$2(0)^{b}$
VBFE	$3(0)^{a}$	$2(0.5)^{b}$	$2.5\ (0.5)^{b}$	$2(0)^{b}$
MECL	$2(0)^{a}$	$1(0)^{b}$	$2(1)^{ab}$	$1(1)^{b}$
AMN	$3(0)^{a}$	$1(1)^{b}$	$1.5(3)^{b}$	$1(2)^{b}$
GE	$3(0)^{a}$	$2.3(1)^{b}$	$3(0)^{ab}$	$2.3(1)^{b}$
CLDN4				
MECL	$1(0)^{a}$	$0(0)^{b}$	$0(1)^{ab}$	$1(0)^{a}$

CLDN1, ZO1 and CLDN4 proteins across MFI regions in control and PRRSV2 infected groups of fetuses. Superscripts indicate statistically significant differences within main effect (P <0.05).

Conclusions and Discussion

Differences were observed between control and PRRSV2 infected fetuses. Only one difference was observed among the PRRSV2 infected groups, where CLDN4 is lower expressed in IUGR compared to MEC fetuses in the MECL. This suggests that PRRSV2 infection alters the integrity of TJ proteins at the porcine placenta. The results of this study will enhance our understanding of the biology of transplacental PRRSV2 infection and may enable strategies to prevent or impede fetal infection.

Acknowledgments

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Investigation of ORF5 and ORF7 gene sequence variability of three live attenuated PRRS vaccines used in large scale swine breeding farms

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Introduction

Given the intrinsic properties of the porcine reproductive and respiratory disease virus (PRRSV), it was considered to be important to analyse the epidemiological observations of the extensive vaccinations and the data provided on the shedding, stability and possible modification of the vaccine strains. The purpose of our study was whether it is possible to determine the presence of a vaccine strain in an infected vaccinated herd with high probability based on ORF5 and/or ORF7 PRRSV gene sequence analyses.

Materials and methods

Three live attenuated PRRS vaccines (Porcilis PRRS (manufacturer: MSD AH);, Unistrain PRRS (former: Amervac), (manufacturer: Hipra); Reprocyc PRRS EU (manufacturer: Boehringer Ingelheim) used in the official PRRS eradicating programme in Hungary were tested. These vaccines have been applied in Hungary since 2002, 2004 and 2015, respectively. Our studies were carried out in large scale breeding swine farms in Hungary, which were in the process of eradication due to their PRRSV infection and were immunized technologically with live virus vaccines. Serological (ELISA) and virologic (PCR) assays were performed in different age groups of these populations, and PRRSV ORF5 gene was sequenced from positive samples. In case of failure, ORF7 was sequenced. Sequence analysis was performed using the "similarity network" diagram in order to identify the closest, most similar sequences to the virus in each vaccine. Subsequently, the percentage similarity of these sequences to the ORF5 and ORF7 of the PRRSV strain of the applied live virus vaccine was evaluated. Epidemiological and immunization protocol data were linked to the sequence.

Results

Analysis of ORF5 and ORF7 sequences is shown in table 1.

Similarity of the PRRSV ORF5/ORF7 sequences found in the assays to the PRRSV sequences in the live virus vaccines tested

All investigated ORF5 sequences:	2342					
Vaccine name	Porcilis	%	Amervac	%	Reprocyc	%
similar in 100 %	338	53,4%	4	2,4%	3	8,6%
similarity is between 99-100 %	269	42,5%	62	37,3%	11	31,4%
similarity is between 98,0-99,0 %	26	4,1%	100	60,2%	21	60,0%
TOTAL	**633**		**166**		**35**	
% of total analysed ORF5 sequences	**27,0%**		**7,1%**		**1,5%**	

All investigated ORF7 sequences:	469					
Vaccine name	Porcilis	%	Amervac	%	Reprocyc	%
similar in 100 %	28	59,6%	5	16,7%	0	0,0%
similarity is between 99-100 %	15	31,9%	13	43,3%	0	0,0%
similarity is between 98,0-99,0 %	4	8,5%	12	40,0%	6	100,0%
TOTAL	**47**		**30**		**6**	
% of total analysed ORF7 sequences	**10,0%**		**6,4%**		**1,3%**	

Conclusion and Discussion

In our experience, the ORF5 gene of the **Porcilis® PRRS vaccine** virus strain has been consistently stable irrespective of the way the vaccine virus enters the body of the pig (vaccination or "infection" after vaccination). If a PRRSV ORF5 or ORF7 sequence is at least 98% similar to the Porcilis® PRRSV strain, it is safe to say that a Porcilis® PRRSV strain has been detected. Care must be taken with Porcilis® PRRS vaccine strain ORF7 gene sequence as it is 100% identical to ORF7 of the Lelystad PRRS strain. In the case of the **Unistrain® PRRS** (Amervac®) vaccine, genetic changes occur more often. The limited number of data available does not allow a similar conclusion to be drawn for Boehringer Ingelheim live virus vaccines yet.

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Reduction of influenza A virus prevalence in pigs at weaning using surveillance and custom made vaccines

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Introduction

Vaccination is the most common intervention to control influenza A virus (IAV) infections in pigs. However, the high genetic and antigenic diversity of IAV often reduces the effectiveness of vaccine control programs. Both commercially licensed and custom-made vaccines can be effective at reducing IAV prevalence at weaning but their effectiveness depends in part on the antigenic matching of the vaccine virus with the wild-type virus circulating in the pigs. In this study, we evaluated a production company driven strategy to reduce levels of IAV at weaning by regularly updating the custom-made vaccines with circulating IAV strains found as part of their surveillance system.

Methods

This case report included data collected for 2.5 years (July 2016-January 2019) from 35 farrow-to-wean farms belonging to an integrated production system in North Carolina. Vaccines were administered seasonally (fall and spring) to all dams in the farm at once (mass vaccination) and/or pre-farrowing. IAV presence in the sow farms was determined by monthly IAV rRT-PCR testing of nasal wipes collected from litters at weaning. Genetic sequences from the sow farm IAV isolates were compared to the IAV isolates of the vaccine used on those sow farms. Specifically, the similarity (% homology) between the hemagglutinin (HA) sequences of the circulating IAV strains and the vaccine IAV strains was determined by pairwise alignment of the translated protein sequences and key amino acid comparison.

Results

Out of the 35 farms, 28 (80%) tested positive throughout the study, and out of 8,352 rRT-PCR tests, 481 (5.75%) were positive and 68 yielded an HA complete sequences. Fifty-four IAV subtype H1 (22 H1-δ, 28 H1-γ, and 4 H1-pdm clades) and 14 subtype H3 (12 IV-A and 2 IV-B clusters) were identified. Custom-made vaccines were updated 3 times throughout the period of this study and included a total of 8 strains. The overall HA amino acid similarity between circulating strains and each correspondent vaccine strain ranged from 95% to 99% while the similarity of the HA antigenic sites ranged from 0% to 71%. IAV herd prevalence decreased from 40% (14/35) at the beginning of the program to 2.9% (1/35) when the study finished. A reduction in the number of positive samples was identified after vaccination in two out of the five vaccine administration periods.

Discussion

In this case report, we documented the decrease of IAV infections at weaning in an integrated production company that uses a systematic approach to monitor, select, and update IAV strains in their custom vaccines. Having a long term approach to the control of IAV is effective but requires the on-going characterization of IAV strains and frequent vaccine strain updates.

Role of nurse sows in the transmission of influenza A virus in pigs prior to weaning

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Introduction

Control of influenza in breeding herds is challenging due to the on-going circulation of influenza A virus (IAV) in the herds. We have identified the use of nurse sows (NS) as a factor that contributes to the persistence of influenza infections. Nurse sows are used to ensure adequate feed intake of piglets to minimize piglet pre-weaning mortality and its use is a common practice in the US swine industry. Transmission of IAV from nurse sows to adopted pigs has been established experimentally. However, the importance of this route of transmission under field conditions has not yet been elucidated. In this study, we evaluated the impact of nurse sows on the transmission of IAV under field conditions.

Methods

A cohort study to test IAV status in nurse and control sows and their corresponding litters, was carried out. We enrolled 184 sows (94 NS and 90 control sows) selected from 3 farrow-to-wean farms with a history of IAV infection. NS were selected at weaning and moved to rooms containing younger pigs to be adopted. Once NS were enrolled, a control sow that was rearing her biological litter located in the same room where the NS was located was also enrolled in the study. Nurse and control sows were sampled using udder skin wipes and oral swabs at the time of enrollment and again at weaning. Six pigs from each litter were sampled conveniently by collecting oral swabs at enrollment, 2 days post-enrollment (2DPE), 4 days post-enrollment (4DPE), at 14 days of lactation (14DL) and at weaning (WA). All samples were tested for IAV by rRT-PCR. Oral swabs collected from pigs were tested in pools of 3 (2 pools per litter). Litters were categorized as positive when at least one pool was IAV rRT-PCR positive.

Results

At enrollment, 76% (69/91) of udder wipes and 3% (3/89) of oral swabs from NS were positive by rRT-PCR compared with 23% (21/92) of udder wipes and 0% (0/85) of oral swabs from control sows. Of the 94 control litters sampled, 11.7%, 14.9%, 22.9%, 46.8% and 63.9% tested rRT-PCR IAV positive at enrollment, 2DPE, 4DPE, 14 DL and at weaning, respectively. Corresponding prevalence for NS litters were 12.2%, 30.2%, 36.9%, 59.4% and 56.4%. The odds of IAV positivity were significantly higher ($p<0.05$) for litters from NS 2 DPE (odd rations (OR) = 6.13), 4 DPE (OR= 5.5) and 14 DL (OR=3.7). However, proportion of positive samples at weaning was similar in control than in nurse sow litters. Moreover, approximately 18% of the control sows and 11% of NS that tested IAV negative in oral swabs at enrollment, tested IAV positive at weaning.

Conclusions and discussion

Litters adopted by nurse sows were more likely to become IAV- infected shortly after adoption, but at weaning, there were no differences in IAV status between litters of nurse and controls sows. This study supports the role of nurse sows in the transmission and persistence of IAV during the pre-weaning period under field conditions.

Non-Thyroidal Illness Syndrome (allostatic hypothyroidism) following PCV2 infection

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Introduction

The thyroid hormones (TH), triidothyronine (T3) and thyroxine (T4), are essential for normal growth and development. Although tightly regulated at multiple levels along the hypothalamic-pituitary-thyroid axis, an allostatic state of hypothyroidism referred to as Non-Thyroidal Illness Syndrome (NTIS), can develop during prolonged and/or severe disease, stress and starvation. We have recently reported that PRRSV2 infection induces NTIS in post-natal and fetal pigs (1, 2). The aim of this study was to determine if PCV2 infection also induces NTIS.

Materials and Methods

Six-week-old pigs were challenged with $10^{4.0}$ $TCID_{50}$ PCV2 at the University of Nebraska following the decay of maternal antibodies(3). Sera collected weekly from 0 to 28 days post inoculation (DPI) from 12 pigs with either high ADG (>0.6 kg/d) and 9 pigs with low ADG (<0.35 kg/d) were provided. T3 and T4 levels were measured in archived porcine sera using commercial RIA kits (MP Biomedicals). Analyses: Changes in TH levels were assessed at each timepoint relative to pre-infection levels using the Signtest, and between ADG group using the Ranksum test. Pearson's correlation determined the strength of association between levels of TH and viremia, previously measured using RT-qPCR(3).

Results and Discussion

All pigs developed viremia (Fig 1) with higher levels noted in low versus high ADG groups on 21- and 28-days post inoculation (DPI). There was a moderate to strong correlation (0.0003<P<.05)between levels of TH and PCV2 viremia starting 14 DPI (Table 1).

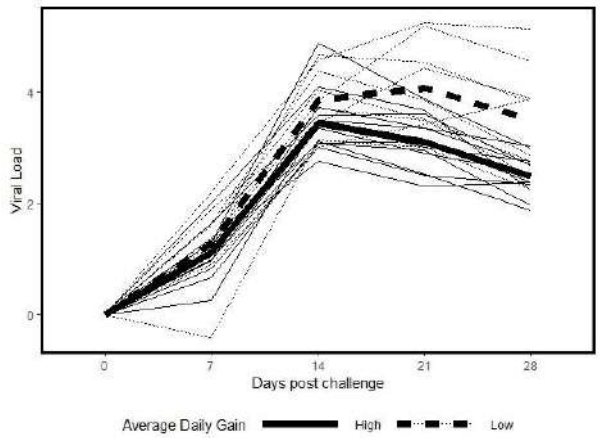

Figure 1. PCV2 DNA levels in sera of viremic group after experimental PCV2 challenge.

Table 1. Negative correlations between T3/T4 levels and PCV2 viremia

PCV2 viremia	T3			T4		
	14	21	28	14	21	28
Day 14	-.41	-.48*	-.36	-.37	-.41	-.34
Day 21	-.49*	-.69*	-.56*	-.26	-.53*	-.55*
Day 28	-.40	-.71*	-.57*	-.17	-.52*	-.55*

*significant at P<0.05; darker = stronger correlation

T3 levels decreased significantly from 0 to 14 DPI in the Low ADG group and trended in the same direction at 21 and 28 DPI but were unaltered in the High ADG group (Fig 2A). T4 levels did not differ by time (Fig 2B). Differences in TH levels were noted between High and Low ADG groups after 14 DPI (Fig 2A and B).

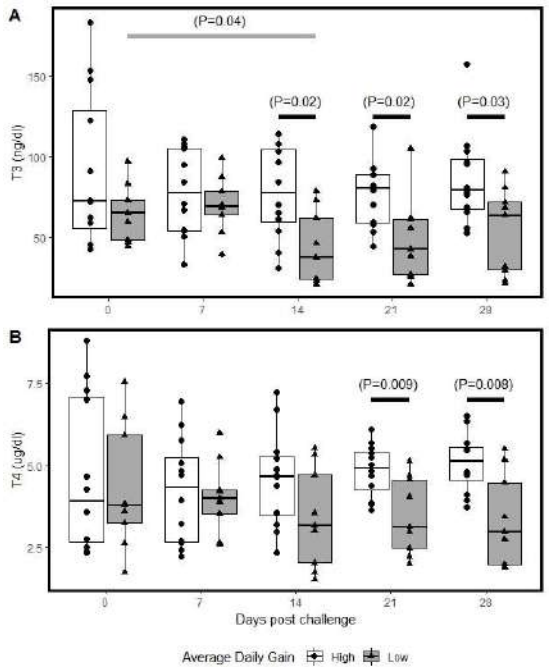

Figure 2. T3 (A) and T4 (B) levels in sera following PCV2 challenge in High and Low ADG pigs

Conclusion

A proportion of pigs who show reduced growth following PCV2 infection also experience hypothyroidism consistent with NTIS.

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Occurrence of influenza A virus subtypes in Brazil

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Introduction

Even after one decade of the first report of pandemic H1N1 (H1N1pdm) influenza virus (IAV) in swine, the disease is highly prevalent in pig farms. Currently, IAV is considered one of the most important primary pathogens in swine respiratory infections in the country (4). IAV is a fast evolving RNA segmented virus which is prone to reassort and accumulate point mutations. Genetically and antigenically diverse H1N1, H1N2 and H3N2 IAVs circulate in pig's farms worldwide with limited cross-protection among distinct virus subtypes and lineages (1). Human seasonal-origin H1N1, H1N2 and H3N2 IAVs, along with H1N1pdm, are widespread in pig farms located in several Brazilian states (3).

The aim of this study was to evaluate the occurrence of IAV in swine from 2017 to 2020 and to update information about IAV in Brazil.

Materials and Methods

From January of 2017 to February of 2020, 1349 nasal swabs and/or lung tissue samples were collected from pigs showing respiratory clinical signs and submitted to Laudo veterinary laboratory for IAV testing. Pig samples were from nursery and finishing farms located in four Brazilian states (Rio Grande do Sul, RS; Santa Catarina, SC; Paraná, PR and Minas Gerais, MG). Viral RNA was extracted using PreAmp (Simbios) kit and tested for IAV/M gene by RT-qPCR (5). IAV positive samples were submitted to viral isolation in SPF-embryonated chicken eggs and to amplification of hemagglutinin (HA) and neuraminidase (NA) genes by RT-qPCR (2).

Results

From 1349 samples tested by RT-qPCR, 473 (35.1%) were positive for IAV. Sixty-four (64) IAVs were isolated in eggs and subtyped by RT-qPCR (Table 1). H1N1 was detected in 20 (31.3%) samples, followed by H1N2 in 17 (26.6%) and H3N2 in 12 (18.8%) samples. Partial subtyping was determined for 15 (23.4%) samples (HxN2: 10 samples; HxN1pdm: 3 samples; huH1Nx: 1 sample; H1pdmNx: 1 sample). The RT-qPCR employed for IAV subtyping is also able to differentiate the IAV lineages. Therefore, 19 out of 20 H1N1 were of H1N1pdm virus lineage. One H1N1 virus was detected with primers and probe designed for an H1N1 virus derived from human seasonal IAV that circulated in late 2000s (2). Novel reassortant H1N2 virus with H1 gene of H1N1pdm was detected in four isolates from SC state. Moreover, co-infection with two virus subtypes was detected in six out of 64 (9.4%).

Table 1. Subtyping results for IAV positive samples collected from 2017 to 2019.

Subtypes determined by RT-PCR	States				
	RS	SC	PR	MG	Total
H1N1pdm	4	8	4	3	19
huH1N1	1	0	0	0	1
huH1N2	2	4	2	5	13
H1pdmN2	0	4	0	0	4
huH3N2	4	2	2	4	12
Partial subtyping	2	7	1	5	15

Conclusions and Discussion

The present study shows the wide circulation of H1N1, H1N2 and H3N2 virus subtypes in pig farms located in four Brazilian states, representing 79.9% of Brazilian pork production. After one decade of its emergence in pigs, H1N1pdm is still causing outbreaks in swine. Previous studies showed the predominance of H1N1pdm in swine since 2009 (3,4), confirming its high fitness in swine. Partial subtyping of 23.4% samples possibly reflects the need to update primers to follow IAV changes observed recently. Following the emergence of H1N1pdm, novel reassortant H3N2 and H1N2 viruses arose in swine (3) and, since 2015 H1pdmN2 virus has been detected in swine in Brazil.

Considering these results, the IAV dynamics and the mutation ability of the virus, monitoring of influenza in swine is important to understand and control the disease more effectively.

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Efficacy of Ingelvac Provenza™ against contemporary H3N2 strains

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Introduction

Multiple influenza subtypes as well as antigenic and genetic variants within subtypes continue to circulate in the swine population in the US. Currently available killed influenza virus vaccines do not provide adequate cross-protection against multiple antigenic variants of SIV in the field [1].

Ingelvac Provenza™ (Boehringer Ingelheim Vetmedica USA, Inc., Duluth, GA) is an intranasally administered live attenuated influenza vaccine (LAIV) that can be given to newborn pigs. The objective of this study was to confirm the efficacy of Ingelvac Provenza™ against contemporary H3N2 strains.

Materials and Methods

The study was performed as a laboratory challenge study involving 80 3-week-old old pigs that were divided into four groups and housed within two rooms. One of the rooms was intranasally vaccinated with Ingelvac Provenza™ and the other room was given a placebo. Just prior to challenge the pigs were rehoused so that each room had half vaccinated and half placebo pigs. Animals of one room were inoculated with (A/swine/SD/2019015191-D/2019/H3N2) and the other were inoculated with (A/swine/OH/A01354299/2017/H3N2). The pigs were observed for 21 days after challenge with the collected data parameters listed in Table 1.

Table 1: Study Design

Group	Room	N	Provenza Day 0	Challenge on Day 14	Parameters (days 1- 21 post challenge)
1	1	20	yes	A/swine/ SD/A0243	Viral shedding
2	1	20	no	0908/2019 /H3N2	(VI, PCR) in NS and BALF;
3	2	20	yes	A/swine/ OH/A0135	Body temperature; Clinical signs;
4	2	20	no	4299/2017 /H3N2	Body weight

Results

Both H3N2 strains resulted in distinct coughing in both placebo treated groups (Figure 1) which was most evident during the first ten days following challenge. Both vaccinated groups exhibited significantly less cough following challenge.

Viral shedding as measured by nasal swab (NS) PCR was distinctly higher over time in the non-vaccinated challenged groups compared to the respective vaccinated controls (Table 2).

Figure 1: Course of coughing

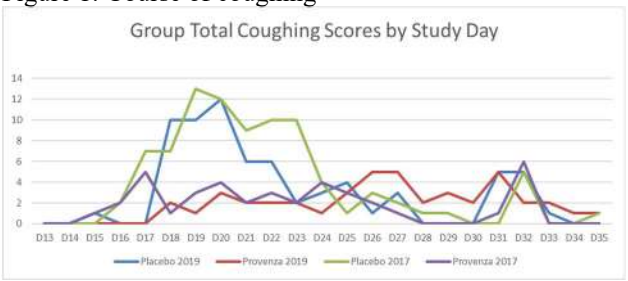

Table 2: Number of nasal swab PCR positives per group and study day

Group	D1	D2	D3	D4	D5	D6	D7	D8	D10	D14	D21
1	0	5	6	11	0	0	0	1	6	3	0
2	19	20	20	20	20	11	3	3	5	1	1
3	13	19	20	14	14	1	0	2	0	0	0
4	15	20	20	20	20	15	4	8	0	0	0

Conclusions and Discussion

The antigenic diversity of contemporary and emerging IAV strains is a challenge for effective IAV-S control.

The current study demonstrates that Ingelvac Provenza™ is efficacious in reducing clinical signs (coughing) and viral shedding against two contemporary H3N2 strains. The results confirm previous findings that both magnitude and frequency of shedding are reduced by vaccinating piglets with Ingelvac Provenza™.

With currently available tools that include vaccination of pigs with Ingelvac Provenza ™, success against IAV-S is achievable.

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Evaluation of bovine viral diarrhea virus transmission by the transplacental route in pregnant gilts experimentally inoculated

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Introduction

Bovine viral diarrhea virus (BVDV) has two genotypes, I and II, and are part of the genus *Pestivirus*, as well as classical swine fever virus (PSC) and border disease. These viruses were reclassified as *Pestivirus* A, B, C and D respectively, and the newly described atypical pestiviruses reclassified as *Pestivirus* E to K (1). Because they belong to the same genus, they may present antigenic and genetic similarity (2). Some of these viruses share the ability to infect swine and other domestic species (3). It is described that BVDV infections in pigs can trigger reproductive changes, such as the birth of low viability piglets, occurrence of mummifieds and abortion (4), besides the birth of piglets with persistent congenital infection (5). Based on the information above, the aim of this study was to experimentally inoculate pregnant gilts with BVDV-2 in the final third of gestation, and evaluate the action of the virus not only in the reproductive system but also in the litters.

Materials and Methods

Eight pregnant BVDV-free gilts on the 105th to 110th day of gestation were selected and allocated into two groups: the challenged one (G1; n=6), in which each female received 15 mL of BVDV-2 suspension in EMEM (Eagles Minimal Essential Medium) by the oronasal route and the control (G2; n=2), which received only the sterile EMEM. At birth, fragments of umbilical cord and placenta of all gilts were collected. Weekly, all piglets born underwent blood samples collection to obtain whole blood and serum up to 21 days of life, Serum samples were evaluated by virus neutralization test (VN). Whole blood, umbilical cord and placental fragments were submitted to RT-PCR test to detect viral RNA.

Results

Viral RNA was not detected in blood, umbilical cord and placenta samples, as well as antibodies against BVDV-2 in serum samples during the different days of collection. The control animals were negative in all tests.

Conclusions and Discussion

According to the data found, no transplacental transmission of BVDV-2 was observed on experimentally inoculated gilts. As BVDV is a ruminant pestivirus little adapted to swine, the immune system of gilts may be able to eradicate the infection with BVDV, and the sistemic viral load may not have been enough to overcome the transplacental barrier. .

Regarding the virus neutralization results, previous studies showed that gilt seroconvertion against BVDV was 20 days (6), varying between 12 and 33 days (7). Taking into account that: (i) females were challenged near delivery; (ii) the lactational period was 21 days; (iii) immunoglobulins in breast milk have lower concentration at the end of lactation (8); (iv) there is individual variation in immune response against BVDV-2; the non-detection of antibodies against the virus, in piglets may be a consequence of this series of factors presented.

The mechanism of evasion of the immune system against BVDV infection is monocytes lysis, which hinders the recognition and subsequent development of a specific immune-humoral response (9). The data are in accordance with that found in the literature, in which experimental infections made in previous studies have shown that BVDV in swine lead to a milder and irregular immune response (6,7,10).

It is believed that the virus was unable to cross a transplacental barrier, which didn't result in the production of anti-BVDV-2 antibodies in piglets throughout the experimental period.

Acknowledgments

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Porcine circovirus type 2 (PCV2) as cause of reproductive failures in a large sow herd in Ukraine

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Introduction

PCV2 is a ubiquitous swine pathogen associated with a number of diseases referred to as PCVD or PCVAD[1,2]. Although PCV2-associated reproductive disease (PCV2-RD) is known since 1999, it may be frequently underdiagnosed under field conditions[2]. This case report describes reproductive failures in a commercial herd and the effect of sow vaccination against PCV2.

Material and Methods

The case herd is a commercial herd of 1000 sows producing feeder pigs (22-25 kg LW), which are moved to other sites for finishing. Sows are farrowing in groups of 40-50 per week. In routine monitoring, the farm is negative to PRRSV. At the end of 2018 the farm reported reproductive losses expressed by increased number of mummified fetuses (figure 1).

Figure 1: I-MR chart (control chart) of percent of mummified fetuses per litter defining the outbreak period from week 51/2018 to week 11/2019. The vertical red line indicates the first mass vaccination with FLEXcombo® in week 3/2019.

Laboratory testing (qPCR) of tissues from mummified fetuses did not confirm the presence of PPV, PRRSV and PCV3. However, high viral loads of PCV2 (Cq values between 13 and 19) were found in tissues. The DNA extracted from the field isolate was sequenced using an in-house test system and confirmed the presence of PCV2d. Other possible differential diagnosis were not further investigated as they were less likely or were ruled out by clinical presentation, missing necropsy findings or treatment history. The suspected diagnosis of PCV2-RD was based on clinical presentation and presence of PCV2d in fetal tissue samples. Vaccination against PCV2 has not been used on farm before this case neither in pigs nor in sows. It was decided to start vaccination of all sows and piglets with Ingelvac CircoFLEX® (in combination with Ingelvac MycoFLEX® as FLEXcombo®). Vaccination protocol started with mass vaccination of the breeding herd and all piglets from 3-10 weeks of age. Routine vaccination of the breeding herd took place every 4 months and piglets were vaccinated at 21 days of age.

Results and Discussion

Next to subclinical forms of the disease, PCV2 infection in breeding females can also cause clinical problems[1,2]. The symptoms of PCV2-RD include those described in this case. With implementation of the vaccination program, a significant improvement could be measured, comparing the data before and after the outbreak, in live-born piglets per litter (p=0.026) and weaned piglets per litter (p=0.005) in favor of the vaccinated sows.

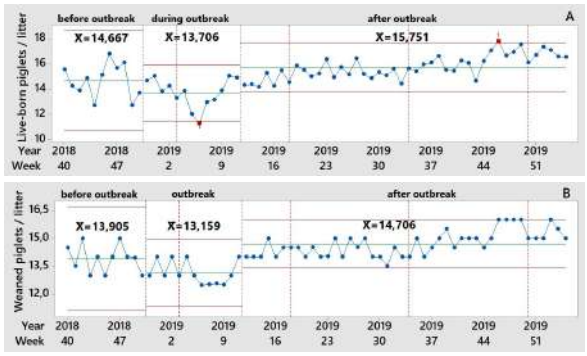

Figure 2A, 2B: I-MR chart of number of live-born piglets / litter (A) and weaned piglets / litter (B). Vertical red lines indicate mass vaccinations with FLEXcombo®.

Conclusion

Vaccination with Ingelvac CircoFLEX® (and MycoFLEX®) was safe at all stages of the reproductive cycle. It was effectively reducing clinical signs of PCV2-RD and improved production parameters of the breeding herd. The causative agent was identified as PCV2d. These findings confirm previously described efficacy of Ingelvac CircoFLEX® against different subtypes of PCV2[3,4].

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Effect of FLEXcombo® vaccination on growth performance of nursery pigs in a large farm in Ukraine

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Introduction

Porcine circovirus type 2 (PCV2) is a ubiquitous swine pathogen associated with a number of diseases referred to as PCVD or PCVAD (Porcine Circovirus (Associated) Diseases)[1]. *Mycoplasma hyopneumoniae* (Mhyo) is the primary agent of enzootic pneumonia, a chronic respiratory disease in swine[2]. Both diseases, among other symptoms, are impairing growing pig performance, especially average daily weight gain (ADG), in infected populations[1,3]. This report describes improvement of average daily weight gain in nursery pigs on a commercial farm that started vaccination in sows and pigs against PCV2 and Mhyo after a clinical outbreak of PCV2-associated reproductive disease (PCV2-RD).

Material and Methods

The case herd is a commercial herd of 1000 sows producing feeder pigs. The farm sold pigs with a mean weight of 23.6 kg live weight during the observational period. Pigs are moved to other sites for finishing. At the end of 2018 the farm reported reproductive losses that were diagnosed as PCV2-RD based on clinical presentation and presence of PCV2d detected by rt-PCR in fetal tissue samples. Vaccination against PCV2 had not been used on farm before this case neither in pigs nor in sows. The farm was also known to be positive for Mhyo, but did never vaccinate pigs against this pathogen. It was decided to start vaccination of all sows and piglets with Ingelvac CircoFLEX® in combination with Ingelvac MycoFLEX® as FLEXcombo®. Vaccination started with mass vaccination of the breeding herd and all piglets from 3-10 weeks of age. Routine vaccination was continued as mass vaccination in the breeding every 4 months herd and in piglets at 21 days of age.

Results and Discussion

Vaccination against PCV2 and Mhyo has previously shown to improve growth performance, e.g. measured in ADG[1,3]. With implementation of the vaccination against PCV2 and Mhyo, ADG of nursery pigs significantly improved (figure 1; $p<0.001$) also in the present case.

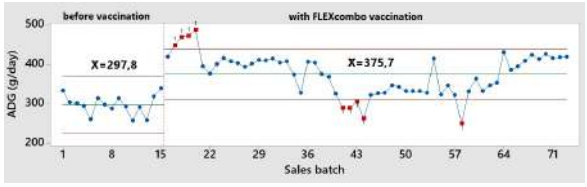

Figure 1: I-MR chart of average daily weight gain in nursery pigs before vaccination against PCV2 and Mhyo and after implementation of FLEXcombo® vaccination. Data was reported for every batch sold.

With the change in ADG the farm was able to sell the pigs one week earlier than before, while maintaining the mean weight at selling ($p=0.252$) (table 1).

Table 1: Mean number of weeks pigs spend in nursery and mean weight at time of sales of pig batches before vaccination and after implementation of FLEXcombo® vaccination.

	before vaccination	with FLEXcombo®
mean weeks in nursery	8.13	7.03
mean weight at sale (kg)	23.0	23.7

Conclusion

Combined vaccination with Ingelvac CircoFLEX® and Ingelvac MycoFLEX® (as FLEXcombo®) significantly improves growth performance in nursery pigs.

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Infection dynamic of porcine reproductive and respiratory syndrome virus in backyard farms in Lima, Peru

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Introduction

In the late 80's and early 90's severe clinical signs in pigs were reported in the United States of America (USA), Canada, Germany, and Japan, among others (1, 2). In 2014 in Peru clinical signs compatible with PRRS were observed in backyard and technified swine farms. In the same year diagnostic laboratory test confirmed the presence of the PPRSV in the Peruvian swine population. The objective of this study was to determine the infection dynamic of porcine reproductive and respiratory syndrome virus (PRRSV) in porcine backyard populations in Peru to better understand the contamination risk these farms pose to technified farms.

Materials and methods

From July to December of 2016, four hundred and Thirty six (n=436) backyard farms were identified in Huaura and Huaral provinces, Peru. All identified farms were located within a 5km radius from one or several technified swine farms. During the farm visit, a physical and clinical inspection of the swine population, and a survey was performed (Table 1). In order to establish PRRS status of the farm, oral fluids from 45 to 70 day old pigs, boars, breeding females and/or other pigs, were taken (Table 2). Oral fluid samples were tested by indirect ELISA (IDEXX Laboratories, Inc., Westbrook, ME, USA).

Table 1. Farm classification parameters.

Animals	IP	**Feed**	**AI**	**EP**	**Manure**
1 - 10	1	swill	-	-	+
11-20	1 - 2	swill/ feed	-	-	+
21-40	1 - 2	swill/ feed	-/+	-	+++
41- 100	2 - 3	swill/ feed	+	-/+	+++
>100	≥3	swill/ feed	+	-/+	+++

IP= Internal personnel; AI= Artificial insemination
EP= External personnel.

Three months after the first sampling, a second sampling from all farms that tested ELISA positive was performed. Five (n = 5) blood samples were taken from animals between 45 and 70 days of age and/or animals that had been introduced to the farm 3 or more days after the first sampling. Samples were pooled and analyzed by real-time PCR (RT-PCR).

Results

From a total of 436 monitored farms, 24.08% (n=105) of the oral fluid samples tested positive to the ELISA test, however no serum pools tested positive to RT-PCR.

Table 2. Number and percentage of PRRS ELISA and PCR positive farms.

Number animals	Number of backyard farms	% ELISA +	% PCR +
1-10	175	14.86	na*
11-20	111	23.42	0
21-40	78	33.33	0
41- 100	57	29.82	0
>100	15	66.66	0

*non applicable, no animals were introduced

Conclusions

The results of this investigation indicate that backyard farms have static and small populations allowing for PRRS stability consequently avoiding continuous replication and excretion of the virus, thus decreasing the contamination risk of neighboring farms.

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Field clinical efficacy of the sow vaccination with Ingelvac CircoFLEX® in PCV2 infected herds in Japan

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Introduction

Porcine circovirus type 2 (PCV2) is known for its wide range of clinical and subclinical manifestations, including reproductive diseases, and has a worldwide impact on swine production (1). In PCV2 subclinically infected sow herds, sow vaccination with commercial PCV2 vaccine has shown a beneficial impact on improving reproductive performance (2,3). The objective of this study was to investigate the efficacy and safety of Ingelvac CircoFLEX® when administered to pregnant sows in Japan.

Materials and methods

Clinical trial was conducted in two commercial farrow-to-finish swine farms in Japan. Totally 186 pregnant sows, 93 sows were randomly allocated to Vaccine group and Control group respectively. Distribution of each gestation stage (1st trimester: service to 5 weeks after service, 2nd trimester: 6-10 weeks after service, 3rd trimester: 11 weeks after service to farrowing) was ensured to be as equal as possible between two groups. On day 0, either Ingelvac CircoFLEX® (Boehringer Ingelheim, Ltd., 1 mL single intramuscular injection) or saline was administered intramuscularly. Clinical observation and monitoring of the local reaction, body temperature and reproductive parameters were conducted during the study. Blood samples were collected every 4 weeks from day 0 to assess PCV2 DNA load by quantitative real-time PCR (qPCR) and antibody titers (SERELISA® PCV2 Ab Mono Blocking, Synbiotics).

Results and discussion

PCV2 circulation was confirmed in both farms from results of qPCR and/or ELISA. No evident clinical signs or increase of the body temperature were observed in any of the sows. Mild swelling was observed at the injection site in one sow in Vaccine group. Farrowing rate was 100% in both groups. Vaccinated sows had significantly less weak-born piglets per litter (-0.3), stillborn piglets per litter (-0.3) and pre-weaning piglet deaths per litter (-0.7) compared to Control group. In addition, normal born piglets per litter (+1.0), suckling piglets per litter (+0.8), weaned piglets per litter (+1.6), average birth weight (+0.1 kg) and birth to wean average daily gain (ADG; +0.05 kg/day) were significantly increased. In Vaccine group, detection rate of PCV2 viremia in the serum of sows and piglets at weaning was 0% and was significantly decreased compared to Control group in one farm while PCV2 viremia was not detected in the other farm.

Table 1. Results of reproductive parameters in Vaccine and Control group

No. of piglets per litter	Vaccine group	Control group	p-value
Total born	11.2	10.9	0.44
Normal born	10.8	9.8	0.007
Weak-born	0.1	0.4	0.003
Stillborn	0.3	0.6	0.047
Initiated suckling	10.9	10.1	0.026
Pre-weaning deaths	0.5	1.2	<0.001
Weaned	10.5	8.9	<0.001
Average birth weight (kg)	1.4	1.3	<0.001
Birth to wean ADG (kg/day)	0.23	0.18	<0.001

Conclusions

This study has demonstrated that injection of Ingelvac CircoFLEX® to pregnant sows in farms where PCV2 circulation was confirmed has a positive influence on prolificacy and vitality of the offspring and reduction of PCV2 viral load. Furthermore, vaccine safety for sows and their offspring was shown by the results of clinical observation, local reaction, body temperature and reproductive parameter. These results strongly support the efficacy of Ingelvac CircoFLEX® on reducing clinical signs of PCV2 associated reproductive failure and PCV2 viremia in sows and their offspring.

Acknowledgments

Kyodoken Institute for Animal Science Research & Development (Kyoto, Japan)

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Impact of a live attenuated influenza vaccine on IAV-S monitoring in a gilt multiplier flow

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Introduction

Replacement gilts are a valuable commodity for the producer and the recipient sow farm. Many sow farms require key pathogen surveillance of gilts prior to delivery. If monitoring results are positive for influenza A virus in swine (IAV-S) by PCR, it is recommended those gilts not be introduced to the sow farm until IAV-S is no longer detected (1). However, delaying shipment until IAV-S samples are negative is costly. This study evaluated the impact of Ingelvac Provenza® (Boehringer Ingelheim Animal Health USA Inc.) administered in neonatal gilts on IAV-S prevalence during the gilt growing period. INGELVAC PROVENZA is an intranasally administered live attenuated influenza vaccine (LAIV) that has been shown to protect against multiple IAV-S strains and decrease nasal shedding, therefore reducing IAV-S transmission in a population (2).

Materials and Methods

All gilts and barrows born at a 2400 head sow farm in the eastern US were vaccinated with an intranasal, 1 ml dose of INGELVAC PROVENZA at one to three days of age beginning February 2018 thru mid-November 2018. Upon weaning, gilts were single sourced to wean-to-finish barns. At approximately 20 weeks of age, prior to shipment of gilts to customer sow farms, each barn had oral fluid (OF) samples collected, approximately one sample per 500 head. OF samples were individually tested for IAV-S by real-time PCR at the University of Minnesota Veterinary Diagnostic Lab (St. Paul, Minnesota). Routine gilt flow IAV-S OF monitoring from 2016 and 2017 (prior to INGELVAC PROVENZA implementation) was compared with OF sampling from 2018 through mid-March 2019 (when vaccinated gilts were being selected). A case was defined as samples collected from a given farm on the same day, and was considered positive if any of the samples submitted were positive for IAV-S via RT-PCR.

Results

This gilt flow had a history of IAV-S circulation. In 2016, the percent of IAV-S positive cases was 5.0% (2/40) and in 2017, cases were 9.09% positive (6/66). In the year 2018, when INGELVAC PROVENZA was initiated in neonatal piglets, there were no IAV-S positive OF samples from the growing gilts (0/29 cases). From January through mid-March 2019, oral fluid samples remained negative (0/19). For the 2 years prior to initiating INGELVAC PROVENZA there were 7.55% IAV-S positive cases (8/106) in this gilt flow. After implementing INGELVAC PROVENZA in all neonates the total number of positive cases in 2018 through mid-March 2019 was 0/48. Additionally, no further respiratory diagnostics were performed during this time, as there were no clinical indications.

Conclusion

The prevalence of IAV-S detected in this gilt flow decreased after the administration of INGELVAC PROVENZA to neonatal piglets. The decrease in influenza virus found by pre-shipment monitoring had a positive impact on both the multiplication system, as gilts could be shipped when scheduled, and also the recipient sow farms, as they received gilts of a desirable age and size. These results showed a reduction of IAV-S in growing gilts in a herd with a history of IAV-S detection, and support more studies to monitor benefits of INGELVAC PROVENZA in multiplier herds.

References

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Maternal antibodies of pigs born to gilts that were vaccinated with a live attenuated influenza vaccine as neonates

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Introduction

Maternal antibodies (MAB) to Influenza A virus (IAV-S) inhibit response to killed IAV-S vaccine but strong MAB do not reduce mortality of pigs when infected with IAV-S (1). Therefore piglets must form their own immune response to IAV-S. This can be achieved using a live attenuated influenza vaccine (LAIV) at an early age such as Ingelvac Provenza® (Boehringer Ingelheim Animal Health USA Inc., St. Joseph, Missouri). The objective of this evaluation was to determine the MAB status of piglets born to gilts which were vaccinated with INGELVAC PROVENZA as suckling pigs compared to offspring of older sows on the same farm where dams receive semiannual mass vaccination with a custom made IAV-S vaccine (CMV; Newport Laboratories Inc., Worthington, Minnesota).

Materials and Methods

Beginning in February 2018, all pigs on a US sow farm were vaccinated intranasally with 1 mL of INGELVAC PROVENZA at 4-7 days of age. A CMV was administered to all females in March 2019. In April and August 2019, serum samples were collected from 60 piglets at 10-15 days of age; half born to dams that were vaccinated with INGELVAC PROVENZA as neonates and half born to older parity sows. Samples were tested using IDEXX Swine Influenza Virus Ab Test (IDEXX Inc., Westbrook, Maine) at Iowa State University, Veterinary Diagnostic Laboratory (Ames, Iowa).

Results

ELISA results showed all samples collected 6 weeks post-CMV were positive with strong MAB (average S/N=0.129 (positive S/N<0.600)) regardless of dam LAIV status. Conversely, serum collected 20 weeks post-CMV from offspring of gilts that received LAIV were 63.4% positive (average S/N=0.483), while piglets born to non-LAIV females were 93.4% positive (average S/N=0.263). See Table 1. The odds ratio that offspring born to an older sow would have positive MAB was 8.11.

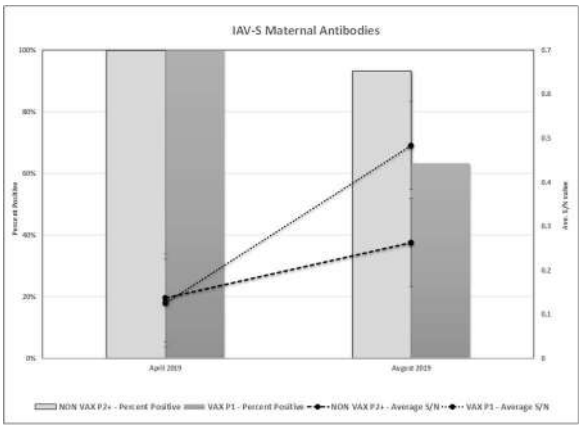

Table 1. Percent positive and average S/N ELISA results (positive S/N<0.600).

Conclusions and Discussion

This preliminary study provides evidence that a LAIV in replacement gilts, a key subpopulation promoting IAV-S transmission at the sow herd level (2), helps prepare piglets to form their own immune response to IAV-S.

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PCV2 and PCV3 viremia under PCV2d field exposure in PCV2 vaccinated and unvaccinated animals

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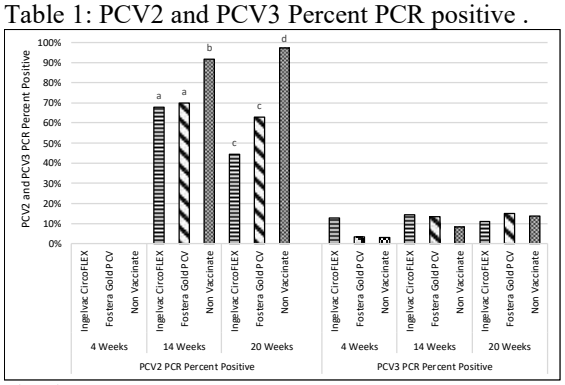
Introduction:
PCV2 vaccines have historically contained PCV2 genotype "a", however recently a vaccine has been introduced to the market which contains PCV2 genotypes "a and b". In addition, the role of PCV3 in growing pigs is largely a mystery. The objective of this study was to provide novel longitudinal data on PCV2 and PCV3 viremia combined with PCV2 vaccine comparison in PRRS and *Mycoplasma hyopneumoniae* negative pigs from wean to market.

Materials and Methods:
Ninety-three barrows were weaned at 18-22 days of age and each pig was randomly allocated to a treatment using a systematic blocked design and assigned to a pen so all treatments were comingled in each pen. At one week post weaning, one of three treatments was administered to each barrow: Treatment 1 - one dose of CircoFLEX® (Boehringer Ingelheim Animal Health USA Inc., Duluth, GA) administered 1 mL per label (n=31); Treatment 2 - one dose of Fostera® Gold PCV (Zoetis, Parsippany, NJ)) administered 2 mL per label (n=30); or Non-vaccinates - no PCV2 vaccine administered (n=32). Serum was collected from all pigs in each group at 4, 14, and 20 weeks of age. Oral fluids were collected at the same time points. Tissues were collected from substandard animals at 14 and 16 weeks of age. All samples were tested at Iowa State University, Veterinary Diagnostic Lab (Ames, IA).

Results:
All pigs were negative for PCV2 at vaccination. At 14 weeks of age, 67.9% of CIRCOFLEX and 70.0% of FOSTERA GOLD vaccinated pigs were positive for PCV2 PCR while non-vaccinates were statistically different with 91.7% positive. At 20 weeks of age, just prior to market, PCV2 viremia was 44.5% and 63.0% positive for CIRCOFLEX and FOSTERA GOLD vaccinated pigs respectively while the non-vaccinates were again statistically different with 97.2% positive (see Table 1). Tissue and serum samples sequenced PCV2d only.
PCV3 viremia (CT < 37) was present in all groups at the time of vaccination and continued to slowly climb throughout the growing phase. Peak viremia for PCV3 was reached at the last sampling with 13.27% of all animals positive. In regards to percent PCV3 positive, there was no statistical difference between PCV2 vaccinated and non-vaccinated animals (see Table 1). Interestingly, four of the ninety-three pigs (4.30%) were positive for PCV3 at each collection from weaning until market.
Serum and oral fluids confirmed that pigs remained negative to PRRS and *Mycoplasma hyopneumoniae* throughout the study.

Table 1: PCV2 and PCV3 Percent PCR positive .



a-b, c-d Differing superscripts denote statistical differences ($p < 0.05$) within each time point sampled. Analysis was done using logistic regression model, using the Bonferroni correction for multiple comparisons.

Conclusion:
This trial provides evidence that current PCV2 vaccines containing genotype "a" or combination of genotypes "a and b" provide equal reduction of viremia to PCV2 genotype "d". Both CIRCOFLEX and FOSTERA GOLD vaccines significantly reduced PCV2d viremia compared to the non-vaccinates, similar to previous studies involving PCV2a and PCV2b challenge.
No correlation was observed between PCV2 and PCV3 viremia. Neither PCV2 vaccine product provided viremia reduction of PCV3 compared to non-vaccinates. An interesting finding from this study is that a small percentage of the population is persistently infected with PCV3 from wean to market.

Histopathologic lesions associated with *in situ* detection of Porcine Circovirus type 3 (PCV3)

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Introduction

The pathogenesis of the novel Porcine Circovirus type 3 (PCV3), identified in 2016, is still unclear, mainly due to unsuccessful attempts at the isolation of this virus. Therefore, the real role of PCV3 with clinical diseases is still unclear. Initial reports have described PCV3 as endemically distributed in the swine population-based on PCR detection. However, the association between virus detection and histopathological lesions is still to be elucidated. The objective of this study was to investigate the histopathologic lesions associated with the detection of PCV3 by RT-PCR in tissue homogenate and by *in situ* hybridization (ISH) in histological lesions.

Materials and Methods

Based on the first description of PCV3, samples with histological lesions and detection of PCV3 by RT-PCR were selected from cases submitted to the UMN-VDL from 2016 to 2018. Cases that had at least two tissues (heart, lung, lymph node, and/or spleen) and positive results for PCV3 by RT-PCR in pools of tissue homogenates were selected. Twenty-five selected cases were categorized according to their Ct values by RT-PCR as follow: low Ct (40%, Ct ≤ 20), intermediate Ct (48%, 20≤ Ct< 30), and high Ct (12%, 30≤ Ct ≤39.9). ISH was performed as described previously (1) using a duplex assay kit (Advanced Cell Diagnostics Inc., USA) for PCV2 and PCV3. The mRNA-ISH targeted the *rep* gene of PCV2 and PCV3 (Genbank: KX298474.1 and HQ839721.1, respectively). Two independent blinded pathologists conducted the microscopic assessment of histological lesions and ISH results. The distribution of positive signals was recorded for association with histopathology results.

Results

Histologically, lesions were mostly observed in heart (16/25) and frequently described as myocarditis (9/16), and epicarditis (6/16). Fourteen out of the 25 cases had lung lesions, with interstitial pneumonia being observed in 10 of them. In other tissues, the most common lesion was vasculitis. PCV3 was most frequently detected by ISH in heart sections (21/22). Additionally, *in situ* detection of PCV3 was associated with systemic vasculitis mainly characterized by the presence of the virus in arterial walls and within perivascular inflammatory infiltrates (17/25). PCV2/PCV3 coinfection was not a common finding, with only 20 out of 87 tissue samples being positive for both virus by RT-PCR.

Conclusions

Myocarditis and vasculitis were the most prevalent lesions associated with the *in situ* detection of metabolically-active PCV3. Due to the endemic distribution of the PCV3 virus in swine herds, the presence of histopathological lesions along with the detection of the virus is critical for the definitive diagnosis of PCV3-associated diseases.

Table 1. Distribution of PCV3-ISH positive signals in the present study.

PCV3 ISH		
Tissue	**Nº. of positive samples**	**Site of ISH signal**
Heart	20/21	cardiomyocytes
	13/21	artery wall
Lung	18/21	alveolar septa
	9/21	arteries
	3/21	bronchioles
Kidney	10/13	interstitium
	5/13	arteries
Liver	9/11	peri-portal
	4/11	arteries
Spleen	10/12	white pulp
	5/12	red pulp
	6/12	arteries
Lymph node	5/6	follicle
	1/6	para-cortex
	3/6	artery wall

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Western Canada pdmH1N1 viruses at the human-pig interface

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Introduction

Since the introduction and spread of the 2009pandemicH1N1 (pdmH1N1), this virus has become part of the human seasonal virus trends, demonstrating evolution and repeated cross-hemispheric spread. The pdmH1N1 virus dominated the 2013-14 and 2015-16 seasons for North American humans and pigs. Human vaccines were updated from A/California/07/2009 H1N1 (CA/09) to contain A/Michigan/45/2015 H1N1 (MI/45). This was updated again to A/Brisbane/02/2019 (6B.1A subclade) for the 2019-2020 Northern Hemisphere vaccine [2]. These viruses are defined by amino acids: S74R, S164T, I295V and the particular change S183P [1,2]. The commercial pig vaccine contains CA/09.

Since human-to-pig infections with pdmH1N1 occurs on a repeated and regular basis, it behooves us to make sure that our pig vaccines are protective against the pdmH1N1 strains that are currently circulating in humans [3]. For this reason, we chose to examine the genetic and antigenic evolution of pdmH1N1 in people and pigs; and the cross-protection of the commercial pig vaccine (CA/09) against circulating pdmH1N1 strains isolated from pigs.

Materials and Methods

SURVEILLANCE: Matrix gene PCR assays were performed on samples collected from pigs on farms with previous influenza activity and farms with acute respiratory outbreaks. One positive (Ct<38) pool was subtyped and virus isolation was attempted on at least one pool with Ct<30. Hemagglutinin gene sequencing was completed on isolates (1/subtype/submission). Phylogenetic analysis performed using Mega6 software [4-7] included published Canadian swine sequences, along with 200 randomly selected American human strains from 2017-18 and 2018-19 seasons (fludb.org).

ANTIGENIC SITE ANALYSIS: The key antigenic sites recognized for swine H1 viruses were examined [8]. Additionally, the human antigenic sites used to classify 6B.1 clade and 6B.1A subclades were examined to aid in the selection of viruses for serologic assays [1,2].

VACCINES: Treatment group 1 was vaccinated with FarrowSure and FluSure pandemic (Zoetis Canada) in the same syringe. Treatment Group 2 was vaccinated with both vaccines in two separate syringes. The vaccines were given twice, 30 days apart and serum was collected prior to the first vaccination and 30 days after second vaccination. VIRUSES: CA/09 (ATCC, VR-1894), A/swine/Manitoba/SD0311/2018 (SD311), and A/swine/Manitoba/SD0398/2019 (SD398).

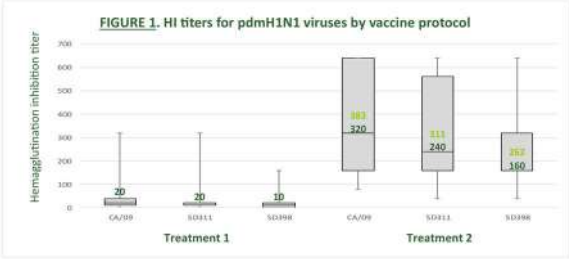
SEROLOGY: The sera were treated with receptor destroying enzyme and Hemagglutination Inhibition assays were performed using Turkey RBCs.

Results and Discussion

SURVEILLANCE: The 2018-19 December/January peak of pdmH1N1 in North American humans was accompanied by a similar peak of detection in pigs. The pig viruses were genetically similar to those found in North American humans who had no pig exposure during the same time period.

As in previous years, the 2019 human peak of pdmH1N1 was followed by a surge in H3N2 viruses. This trend was also found in pigs with the pig H3N2 viruses along with the regionally dominant Alpha3 H1N2 viruses in fall 2019.

SEROLOGY: The HI titers demonstrated a significant difference between treatment 1 and treatment 2 for all 3 strains ($P < 0.01$). This demonstrates the need to keep these two vaccines separate in the pre-farrowing gilt acclimation program. When examining the titers for treatment 2 more closely, there was no significant difference between the 3 virus strains. However, a trend of decline in cross-reactivity was observed (CA/09 vs. SD398; $P = 0.09$); Figure 1.



Although the pdmH1N1 virus has evolved significantly (both genetically and antigenically) over the last decade, it continues to readily infect both humans and pigs, causes a significant respiratory disease burden for both species. The findings here underscore the importance of public education on vaccines and making updated vaccines available for pigs.

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Assessing the value of Lab-on-a-Chip (LabChip)-based real-time PCR assays in oral fluid samples for detecting African swine fever virus as a herd monitoring system

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Introduction

African swine fever (ASF) is a highly contagious and devastating disease of pigs; domestic pigs as well as wild boar, which causes significant economic losses to the pig industry when affected [1]. The virus typically causes hemorrhagic fever with high mortality in domestic pigs and wild boar, whereas infections in African wild suids, such as warthogs and bushpigs, run a nonpathogenic course [2]. Due to the current unavailability of vaccines or treatments for ASF, which is caused by African swine fever virus (ASFV), rapid and reliable detection of the virus is essential for timely implementation of emergency control measures. In this study, a novel Lab-on-a-Chip (LabChip)-based real-time PCR assay was developed for detection of ASFV in oral fluid samples as a herd monitoring system

Materials and Methods

Oral fluid and sera were collected from a commercial wean-to-finish swine farms in VietNam where experienced ASFV lately. Serum was collected from five pigs in 3 pens and parallel oral fluid samples were collected using rope. Thereafter, oral fluid and sera were tested to detect ASFV DNA using LabChip-based real-time PCR system (Veri-Q PCR316 system, Micobiomed Co. Ltd, Korea).

Both spiked and non-spiked oral fluid samples with ASFV (Hanoi_2019) serial tenfold dilutions of the prepared from 10^8HAU/ml to 10^0 HAU/ml).

Figure 1. LabChip_16CH is made of transparent polycarbonate material. It is a lab-on-a-chip (LabChip) product that combines micro mold, mass film bonding technology and microfluidic technology.

Results

The LabChip-based real-time PCR system detected a minimum of 10^0 HAU/ml of the ASFV spiked oral fluid. In the pen no. 1, 5 out of 5 sera were negative and oral fluid was negative in PCR. In the No. 2, 2 out of 5 sera were positive and oral fluid was positive. On the other hand, pen no.3, 0 out of 5 sera were positive and oral fluid was positive.

Conclusions and Discussion

In summary, a novel LabChip-based real-time PCR assay was developed to enable the detection of all possible circulating ASFV in oral fluid. It is suggested that this novel real-time PCR using oral fluid could provide reliable diagnosis and surveillance of ASF complementary sera tests.

Acknowledgments

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Use of endogenous porcine RNA in porcine reproductive and respiratory syndrome virus (PRRSV) RT-qPCR to control for sample addition and degradation

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Introduction

Reverse transcription real time PCR (RT-qPCR) for PRRS is a widely used diagnostic method for PRRSV. However, differences in sample types and quality can have an impact on PCR results, potentially leading to false negative results[1]. The use of an internal sample control (ISC)[2], based on the detection of an endogenous porcine RNA sequence present in the host sample, can help to minimize this problem as the ISC controls for sample addition, extraction and amplification.
This study reports on ISC Cq values using a RT-qPCR for detection of PRRSV in different samples types commonly used for PRRSV diagnostics.

Materials and Methods

A total of 198 samples were analyzed using RealPCR* PRRSV-2 RNA Test (IDEXX Laboratories Inc). The samples originated from different farms and included serum (n=117), oral fluid (n=40), blood (n=6), processing fluid (n=19), umbilical cord and lymph node (n=14) and cell cultured PRRSV vaccine used as a negative control for swine RNA (n=2). Extraction was performed using RealPCR* Magnetic Bead Kit and RealPCR* DNA/RNA Spin Column Kit (IDEXX Laboratories Inc), MagMax™ Pathogen RNA/DNA Kit (Applied Biosystems), QIAmp cador Pathogen Mini Kit (QIAGEN) and taco™ DNA/RNA Extraction Kit (GeneReach). The following real-time PCR instruments were used: Applied Biosystems QuantStudio 5, Applied Biosystems 7500, QIAGEN Rotor-Gene Q and Bio-Rad CFX96 Touch. ISC Cq values were compared across sample types with ANOVA and Tukey test and differences statistically evaluated (α=0.05) with Statistica 8.0 (StatSoft Inc).

Results and Discussion

Mean ISC Cq values for the different sample types evaluated in this study are shown in Table 1 and Figure 1. Internal control amplification curves were recorded for all sample types except for cell cultured PRRSV vaccine samples. Statistically significant differences between mean ISC Ct values for serum, oral fluid and blood were observed when compared with processing fluid and tissue (umbilical cord and lymph node) samples
.

Table 1. Mean ISC Cq values and standard deviations by sample type.

	Serum	OF	Blood	PF	T
ISC Cq	28.3[a] ±3.9	28.3[a] ±3.7	26.0[a] ±3.9	22.7[b] ±3.8	21.1[b] ±3.9

OF, oral fluid; PF, processing fluid; T, tissue.
The values with different superscript differ significantly.

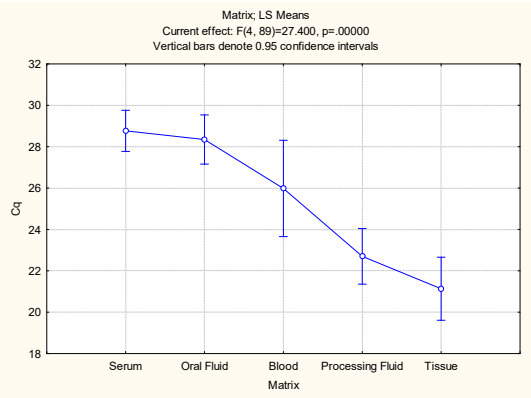

Figure 1. Mean ISC Cq values by sample type. Vertical bars denote 95% confidence intervals.

Serum, oral fluid and blood samples showed similar ISC Cq values and were higher when compared with tissue and processing fluid samples. This may be due to a different concentration of endogenous porcine RNA (ISC target sequence), differences in sample extraction efficiency or to the presence of different level of inhibitors in the different sample types.
In this study, we have characterized mean ISC Ct values for different sample types commonly used in PRRSV diagnostics, stablishing reference values. Comparison with this reference values will help diagnostic laboratories using RealPCR* PRRSV-2 RNA Test to identify sample addition and degradation problems while controlling for extraction and amplification inhibition.

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Influenza A virus infection in nursery pigs in Santa Catarina state, Brazil

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Introduction

In Brazil since 2009, frequent outbreaks of acute respiratory disease in pigs caused by influenza A virus (IAV) are reported. H1N1pdm09, human seasonal-origin H1N2 and H3N2 viruses are widespread in pig herds, where they continue to evolve (1). Most of the studies conducted so far were in growing to finishing pigs. However, porcine respiratory disease complex, such as influenza, are responsible for most of the losses and the use of medications in nurseries. The objectives of this study were to detect the circulation of IAV subtypes and to evaluate the IAV prevalence in swine nurseries located Santa Catarina (SC), the largest pork producer (27.9%) and exporter (51.12%) state in Brazil (2).

Materials and Methods

The study was conducted between June and September of 2018 in nurseries (with a total of 60 thousand piglets) located in the west region of SC State. Nasal swabs and blood samples were collected from 35 to 54 days-old piglets presenting respiratory clinical signs. For sampling, a confidence level of 95%, accuracy level of 5% and a prevalence of 40% were considered (3), totalizing 423 samples.

Nasal swabs were analyzed by RT-qPCR (IAV/M gene) (4) and three IAV positive samples per nursery were subtyped by using an additional RT-qPCR (5). Sera were evaluated for IAV antibodies using an ELISA (InfA Multispecies - CK401- Biocheck) and hemagglutination inhibition (HI) test (6), using as reference viruses the IAVs isolated in pigs in Brazil (H1N1pdm09/ 107-10, H1N2/ 31-11-1, and H3N2/ 28-15-8).

Results

IAV circulation was identified either by detection of viral RNA by RT-qPCR in nasal swabs (67.4%), as well as for the presence of antibodies produced against IAV by ELISA (66.9%), confirmed by the HI results (Table 1). The HI test revealed antibodies in piglets against the H3N2 virus (59/155; 38.0%), H1N1pdm09 (37/155; 23.8%) and H1N2 (5/155; 3.2%). In eight out of 11 nurseries, antibodies for at least two virus antigens were detected. IAV subtyping by RT-qPCR has detected H3N2 virus in six nurseries and H1N1pdm09 in two nurseries. The IAV subtype was not determined in samples from three nurseries (Table 1).

Table 1. IAV virus and antigen detection by ELISA, RT-qPCR and HI test

Herd	Number of samples	ELISA Positive	RT-qPCR Positive	CT range	Subtyping	Hemagglutination Inhibition		
						H3N2	H1N1	H1N2
1	40	31 (75.5%)	18 (45%)	25.63-38.87	H3N2	40-320	40	40-80
2	50	23 (46%)	5 (10%)	35.01-37.96	Not detected	<10	40	<10
3	40	24 (60%)	38 (95%)	21.84-37.46	H3N2	40-320	<10	<10
4	32	27 (84.4%)	29 (90.6%)	26.48-36.52	Not detected	80-640	40	<10
5	40	39 (97.5%)	34 (85%)	20.55-37.72	H3N2	40-80	40-80	<10
6	26	15 (57.7%)	24 (92.3%)	17.92-36.60	H1N1pdm	160	40	<10
7	33	22 (66.7%)	25 (75.8%)	17.75-38.45	H3N2	<10	80	<10
8	50	47 (94%)	29 (58%)	28.65-37.77	Not detected	40-640	40-80	40
9	40	30 (75%)	40 (100%)	13.69-31.35	H3N2	40-80	40-80	<10
10	40	11 (27.5%)	31 (77.5%)	15.64-33.90	H3N2	40-160	40	40
11	32	14 (43.8%)	12 (37.5%)	20.19-37.99	H1N1pdm	40	80	<10

Conclusions and Discussion

Our results show a high prevalence of IAV in pig nurseries in SC state and H3N2 virus was the most detected subtype. Influenza outbreaks are commonly observed in pig herds in Brazil since the introduction of H1N1pdm in 2009 (7). Previous studies have revealed H1N1pdm09 as the predominant viral subtype circulating in Brazilian pigs in the last years (8,9). However, recently, the detection of H3N2 virus has increasing in farms in Brazil. Our data further reveal that 9.6% (15/155) of the piglets reacted to at least two antigens. Thus, different viral IAVs are circulating in the swine population causing mixed infections and contributing to viral genetic rearrangements.

Our results corroborate the need to understand the evolution of IAV viruses in nurseries to better control the infection and future virus reassortments that may generate new outbreaks.

Acknowledgments

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Analysis of the performance of the animal health surveillance system in the outbreak of swine vesicular disease in the State of Santa Catarina, Brazil

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Introduction

The occurrence of vesicular disease associated with Senecavirus A (1) in the swine commercial regions of Brazil (2,3), which includes Santa Catarina, caused an increase in the number of syndromic notifications to the official state animal health defense service in 2015, in order to rule out the suspicion of foot-and-mouth disease in a region considered free without vaccination (4). In view of the subsequent recurrences and operational disorders linked to the event (5), the objectives of the study were to analyze the official data related to cases of suspected vesicular disease in pigs, and to evaluate the performance of the state animal health surveillance system by comparing performance parameters between investigations in 2015 and years after 2015.

Materials and Methods

Descriptive analyzes were performed on data associated with official procedures to address suspected vesicular disease in pigs in the different regions of the State between May 22, 2015 and March 28, 2019, and three statistical models were used to: i) test the effect of year on the age of the investigated injuries; ii) assess whether there is an association between the year and the type of outcome of the official investigation (discarded case and probable case of vesicular disease, which results in the collection of samples for laboratory diagnosis and interdiction of the affected properties); iii) assess whether there is an association between the year and the detection of Senecavirus A RNA among the laboratory analyzes carried out in the investigations classified as probable cases of vesicular disease.

Results

In the analyzed period, there were 2093 notifications of suspected vesicular disease in pigs to the animal health defense service of Santa Catarina, 1538 (73.5%) of which occurred in 2015, and 555 (26.5%) in subsequent years. After 2015, when compared to the base year, the chances of detecting late vesicular lesions (> 3 days) were similar (increased 1.11 times, but there was no statistically significant association), in view of a scenario in which 55.29% of cases the lesions were classified as late throughout the analyzed period. The variation in the chances was relatively homogeneous among the regional units (Intraclass Correlation Coefficient - ICC = 10.9%), but in the São Miguel do Oeste unit it was significantly lower than the average, as well as in regions with a high ratio of properties with commercial pigs by official veterinarian, compared to the low. The chances of the cases being considered probable increased 32.3 times, but the descriptive analysis of the average period of interdiction of the affected properties decreased. The estimated ICC was 34.9%, and in Campos Novos and Caçador, the chances were significantly higher than the average, while in the Canoinhas unit, the chances were significantly lower. The prevalence of cases with molecular detection of Senecavirus A was 78% lower.

Conclusions and Discussion

The analysis showed an increase in the sensitivity of the surveillance process, but decreased the specificity of the laboratory diagnosis of Senecavirus A in the face of suspected vesicular events in the years after 2015. In order to minimize the operational disturbances of the investigations without compromising the syndromic focus of its vesicular character, it is inferred the importance of standardizing the type of late lesion to be sampled, as well as the association of diagnostic techniques (6,7), to cover the endemicity and mischaracterization that has been guiding the manifestation of Senecavirus A (8).

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Infection of Flt3L-derived DC by different strains of African swine fever virus (ASFV)

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Introduction

African swine fever virus (ASFV) is one of the most devastating pathogens for the swine industry. Macrophages are the main target of the virus, while lymphocytes are severely affected through extrinsic apoptosis (1). However, the interaction with dendritic cells (DC) is largely unknown. The present study aims to assess the susceptibility of Flt3L-derived DC to different ASFV strains with diverse virulence and the modulation of those cells by infection.

Material and Methods

Cells: DC were derived from bone marrow hematopoietic stem cells by Flt3L stimulation *in vitro*. For the present study, conventional DC2 (cDC2) ($CD14^{-}CADM1^{+}MHC\ II^{+}CD172a^{+}$) and $CD14^{+}$ DC ($CD14^{+}CADM1^{+}MHC\ II^{+}CD172a^{+}$) were sorted for testing of susceptibility to ASFV infection.

Viruses: Four ASFV stains including two virulent strains (BA71 and Georgia 2007/1), one attenuated strain (BA71△CD2) and one avirulent strain (BA71V) were used.

Assessment of the infection in DC: Sorted cDC2 and $CD14^{+}$ DC were inoculated (at 37°C) with four ASFV strains at MOI 1.0. Inoculum was removed after 1.5 h and cells were then incubated until 24h post-inoculation (hpi). Replication of the virus was assessed by titration on macrophages (detected by IPMA), by qPCR (cell culture supernatants collected at 24 hpi and cells collected at 1.5 and 24 hpi), and by flow cytometry targeting viral vp72 in permeabilized cells.

Expression of MHC I, MHC II and CD80/86 on ASFV-inoculated cDC2: Surface MHC I, MHC II and CD80/86 expression were assessed on inoculated cDC2 cells at 24 h post-inoculation (MOI 1.0) by flow cytometry.

Endocytic capability of ASFV-inoculated cDC2: Endocytic capability of ASFV-inoculated cDC2 was assessed by uptake of Dextran-FITC (MW 40,000) at 1 mg/ml (2.5 h at 37°C), compared with the uninfected cells.

Results

Both cDC2 and $CD14^{+}$ DC were infected by BA71, Georgia 2007/1, BA71△CD2 and BA71V, as evidenced by the presence of viable viral progenies in the supernatants (Figure 1A), the proportion of vp72-positive cells (Figure 1B), and the increase of intracellular (data now shown) and extracellular viral DNA (shown as genome equivalent copies, GEC) (Figure 1C). Strikingly, the virulent Georgia 2007/1 presented the lowest infection efficiency in both cDC2 and $CD14^{+}$ DC cell types; while for the other virulent strain, BA71, replication was higher in $CD14^{+}$ DC than in cDC2 (Figure 1). The proportion of vp72-positive cells in $CD14^{+}$ DC was generally higher than in cDC2 (Figure 1B).

Virulent strains BA71 and Georgia 2007/1, but not the attenuated (BA71△CD2) or avirulent (BA71V) strain significantly up-regulated MHC I expression on cDC2 (Figure 2). No significant difference was found regarding the expression of MHC II or CD80/86 between infected and uninfected cells. ASFV infection did not affect the dextran-uptake ability of cDC2.

Conclusions and Discussion

1. The preliminary data suggested that cDC2 and $CD14^{+}$ DC were susceptible to ASFV infection regardless of viral virulence.
2. Up-regulation of MHC I in cDC2 seems to correlate with viral virulence, since only BA71 and Georgia 2007/1 produced this effect *in vitro*.

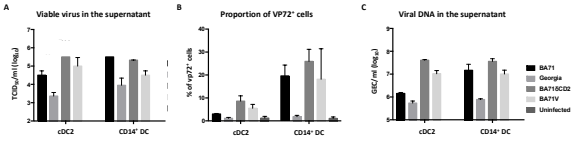

Figure 1. Susceptibility of cDC2 and $CD14^{+}$ DC to different ASFV strains, BA71, Georgia 2007/1, BA71△CD2 and BA71V, at MOI 1 for 24 hours. (A) Viable virus in cell culture supernatant determined by titration on PAM; (B) Proportion of vp72-positive cells analyzed by flow cytometry; (C) Genome equivalent copies (GEC) per ml in cell culture supernatant detected by qPCR.

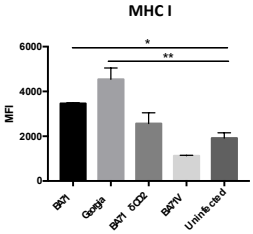

Figure 2. Modulation of MHC I expression on cDC2 by different ASFV strains, BA71, Georgia 2007/1, BA71△CD2 and BA71V, at MOI 1 for 24 hours. Statistical analysis was relative to uninfected group based on MFI (median fluorescence intensity), $*p < 0.05$, $*p < 0.01$.

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Comparison between processing fluids and blood samples for PRRSV monitoring in breeding herds

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Introduction

The clinical evidence of PRRSV infection depends of many factor (1), of those the herd management control is critical (2). Nowadays the most common approaches for herd classifications are based on risk level and the production of PRRS-free weaned pigs is the ideal benchmark (2). A typical monitoring plan is based on 30 blood samples collected from pre-weaned piglets every 3-4 months, with the effective risk to underestimate the prevalence or misclassified positive herds (3). With the purpose to improve the sensibility of the analysis, other diagnostic material were tested and the PF (processing fluids) were validated (3,4,5,6) as an effective and reliable tool. Aim of this study was to asses a comparison between the PRRSV longitudinal monitoring based on blood samples or PF.

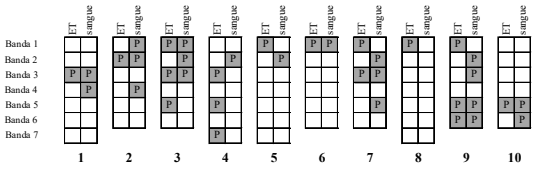
Materials and Methods

PF and blood samples were collected in 10 farms with a 3-week BMS (batch management system), during 5 months of study. PF (not including tails) were collect with standard procedures (5) in a pool of 10-15 litters to have a standard size of the sample, 30 blood samples were after collected from the same batch before weaning and grouped in six pools. RNA purification for all the samples were operated with MagMax Core Nucleic Acid Purification (Applied Biosystem, Foster City, California) by automatic system Biosprint 96 (Qiagen, Hilden, Germany) and PCR real-time were performed with Virotype PRRSV RT-PCR (Indical Bioscience, Leipzig, Germany) following the kit instructions guidelines. Positive benchmark was set under 37 Ct (cycle threshold). Herd status for PRRSV were compared each batch in according to PCR real-time results. The presence of at least one positive sample per batch were sufficient to identify a positive status. Concordance Index was calculated considering K-Cohen statistics.

Results

Average results of PCR real-time showed 32.07 (±2.67) Ct and 2.24 x 10^5 viral copies (± 5.58 x 10^5) for PF and 29.01 (±5.22) Ct with 2.25 x 10^7 (±8.41 x 10^7) viral copies for blood samples. Herd status for PRRSV based on batch monitoring results are shown in Table 1. The calculated concordance was 69.0% with 0.25 of k-Cohen.

Table 1. Comparison of herd status for PRRSV based on PF and blood samples outcome in PCR analysis. Positive (P) sampling were marked.



Conclusions and Discussion

The PF samples showed weaker value of positivity in PCR (lower Ct and viral copies), as already reported in literature (3) because of the diluition effect. Results showed the same PRRSV classification for 45 batches, while 19 batches had a contrasting status, also highlighted by the low K-Cohen index. A deeper analysis of these results disclose the truthfulness of both tests, PF express the status of 3-4 days old pigs; blood samples shows the batch status at pre-weaning age. During the time between the two seampling evets the viremia dynamics can change and the outcomes of the test also. Therefore, PF represent a wider population and allow a more reliable inference. On the other hand, blood samples can be collected from older pigs and a wider temporal distribution.
Finally, PF are a clever and reliable material for a PRRSV monitoring program. As already suggested by other studies (6), our proposal for a more efficient monitoring program is an integrated approach that combines the strengths of both materials.

Acknowledgments

We thank our veterinary colleagues Greta Tavella, Enrico Giacomini, Francesco Vantini, Giovanbattista Guadagnini and all the farmers.

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Detection of influenza A virus in pig farm workers

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Introduction

Influenza A virus (IAV) affects many hosts, including pigs and people, which represents a public health concern[1]. Bidirectional transmission of IAV leads to both disease morbidity and the potential to generate novel IAV strains. US swine herds currently experience frequent outbreaks of acute influenza that can impact both swine and human health. Despite the recognition that bi-directional transmission of influenza virus occurs between people and pigs, little is known about how frequently this transmission or exposure takes place. Therefore, the objectives of this study were to implement a surveillance system at the farm worker-swine interface, quantify how frequently farm workers tested positive for IAV and whether workers have identifiable risk factors that can be predictive of virus introductions into farms.

Materials and Methods

Three sow farms located in the US-Midwest were selected for the study. The farms were representative of US commercial farms with an average of 4,000 sows. The farms had a history of IAV infections and 2 of the farms tested IAV positive at the time to initiate the study. We aimed to recruit swine farm workers that worked with pigs at least 2 days a week. After collecting baseline information, we asked each participant to self-collect a nasal swab before entering the farm and at the end of the working day, after completing the daily chores, twice a week for 8 weeks during the human influenza season as determined by the Centers of Disease and Prevention (CDC). At each sampling points, participants also collected their body temperature using disposable thermometers and answered a short survey regarding the activities performed during the day and whether they had influenza-like illness (ILI).
Pigs were also sampled at three time points during the study. Each time 30 nasal swabs were collected from 20 day old pigs.

Farm worker samples were tested individually with an influenza A specific rRT-PCR test that detects both human and swine IAV's [2]. Samples with a cycle threshold <38 were considered positive and selected for virus isolation on Madin-Darby canine kidney cells[3].
Pig samples were tested in pools of 3 using an rRT-PCR that targets the conserved matrix gene of IAV[4].
The study took place during the 2019 peak of human seasonal influenza (January-March).

Results

There were 34 workers enrolled from the three participating farms and a total of 1027 farm worker samples were obtained. Out of the samples collected, 35 samples (3.4%) tested IAV rRT-PCR positive and out of 34 workers, 20 of them tested IAV positive at least once during the study. However, we did not detect significant risk factor associated with testing IAV positive in the workers.

Conclusions and Discussion

We were able to establish an IAV surveillance system where workers complied with self-sample collection protocols which indicates our ability to perform studies at the pig-human interface in pig farms.

Our results provide evidence that a number of farm workers can test influenza positive when they report to work at swine farms and at the end of the working day and can be indicative of IAV exposure while working with pigs at the farms.

Further characterization of the samples is needed to understand the implications for virus introduction into farms and risk of bi-directional transmission between pigs and people.

Acknowledgments

This study was funded by the National Pork Board

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Successful control of PRRS utilizing oral fluid test in a farrow-to-nursery farm in Japan

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Introduction

Porcine respiratory and reproductive syndrome (PRRS) is a viral disease caused by PRRS virus (PRRSV). Major clinical manifestations are reproductive failure in breeding herd and respiratory disease in growing pigs. In PRRS endemic farms, comprehensive approach such as immunization of breeding and/or growing herd, enhanced biosecurity measures, prevention of viral circulation by controlled pig flow management is crucial to minimize the economic impact of PRRS (1). In 2018, increase of mortality in nursery was observed in a farrow-to-nursery farm in Japan and PRRS was suspected to be involved. This report documents the improvement of mortality in nursery by implementing series of PRRS control measures including PRRS monitoring utilizing oral fluid test, piglet immunization with modified live virus (MLV) vaccine, change of antibiotic treatment regimen and pig flow management.

Materials and Methods

1,100 sows were reared in a farrow-to-nursery farm in pigdense area in Japan. Piglets were moved from farrowing house to nursery at 25 days of age and from nursery to fattening house in a different site at 70 days of age. All-in all-out system was employed in farrowing house and nursery. In November 2018, increase of dead piglets suffering from respiratory disease was observed in nursery. In April and May 2019, wild type (WT) PRRSV gene was detected from serum and lung of piglets with 30-70 days of age. Monthly PRRS monitoring program by oral fluid test including PCR and sequence analysis of ORF5 gene started from June 2019. Oral fluid was collected by a rope method. Monitoring results showed the high frequency of WT PRRSV detection in isolation house where pigs with inadequate growth were gathered and reared. In addition, nursery adjacent to above isolation house showed similar monitoring result.

From June 2019, PRRS MLV combination vaccine (Ingelvac® 3FLEX, Boehringer Ingelheim Animal Health Japan Co., Ltd., 2 mL single intramuscular injection) was administered to piglets at weaning. In addition, tylvalosin premix was added to feed in nursery and pig flow was changed to abolish the isolation house. Monthly mortality in nursery was monitored to evaluate the impact of PRRS control measures.

Results and discussion

Decrease of mortality was observed immediately from June 2019, which indicated the positive impact of PRRS MLV vaccination to piglets and introduction of tylvalosin premix (Figure 1). Results of oral fluid test demonstrated the decrease of WT PRRSV detection (Table 1) and delayed PRRSV infection timing in nursery (Table 2), suggesting the additional positive impact of new pig flow management implemented from August 2019.

Conclusions

This report has demonstrated the positive impact of implementing appropriate PRRS control measures in a PRRS endemic farm. PRRS MLV vaccination to piglets contributed to reducing the viral load. Clinical symptoms were reduced by the diminished PRRSV replication with tylvalosin premix (2) and pig flow management was efficacious on preventing virus circulation. Furthermore, elucidating infection dynamics i.e. frequency of WT PRRSV detection and timing of infection by houses, by continuous PRRS monitoring utilizing oral fluid test is crucial to establish PRRS control strategy.

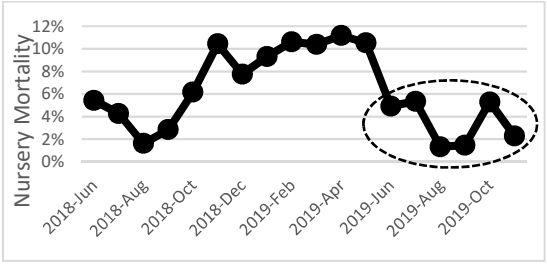

Figure 1. Monthly mortality rate in nursery

Table 1. WT PRRSV detection in nursery in 2019.

Month	Jun	Jul	Aug	Sep	Oct	Nov
Houses with WT PRRSV	3/8	3/8	**1/8**	**1/7**	**1/8**	**1/7**

Table 2. PRRSV infection timing before and after the new pig flow management.

	Average infection timing
Before (Apr-July 2019)	39.7 days of age
After (Aug-Nov 2019)	60.9 days of age

Acknowledgments

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Occurrence of different Swine Influenza virus subtypes in Brazilian herds from 2012 to 2019

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Introduction

Among the respiratory diseases that affect swine production, Swine Influenza virus (SIV) is striking. In Brazil, SIV has been threatening the pork production since the emergence of H1N1 2009 pandemic influenza virus (pH1N1). Since then, pH1N1 appears to have become endemic in Brazilian swine herds, with a higher prevalence compared to other circulating seasonal subtypes of human origin: H3N2 and H1hu (H1N2 and H1N1) (1). A monovalent and whole inactivated influenza A (H1N1) pdm09 vaccine is available in Brazil since 2014. However, there are concerns about protection of the herds, since studies have pointed limited vaccine cross-protection against heterologous subtypes (2). Thereby, monitoring SIV subtypes circulating in swine population is essential. The aim of this study was to evaluate the occurrence of SIV subtypes from routine diagnostic samples, collected between 2012 to 2019.

Materials and Methods

From 2012 to 2019, a total of 167 samples of lung tissues, nasal swabs and cellular culture, collected from pigs with respiratory clinical signs, and positive for SIV matrix gene by RT-PCR (3), were SIV subtyped (pH1N1, H3N2 and H1hu) by a Nested RT-PCR (4). Samples were from swine herds from the South and Southeast regions of Brazil and were analyzed at the Laboratório de Pesquisa em Virologia Animal of the Escola de Veterinária, UFMG. From years 2012-2013, 17 samples were tested; 49 in 2014-2015; 26 in 2017-2018; and 75 in 2019. A total of 1,631 serum samples, collected from pigs belonging to non-vaccinated herds were analyzed for the presence of antibodies against pH1N1 and H3N2 by HI test (3). Of those serum samples, 263 were collected in 2017; 677 in 2018 and 691 in 2019.

Results

The percentage ratio of SIV subtypes and samples positive for H3N2 and pH1N1 antibodies are shown in Figure 1 and 2, respectively. Through molecular analysis, a higher occurrence of pH1N1 between 2012 and 2015 was observed. However, H3N2 predominated in 2017-2018 and H1hu in 2019, followed by pH1N1. The serological data was similar to the molecular analysis, showing cyclicality among the subtypes of SIV between 2017 and 2019.

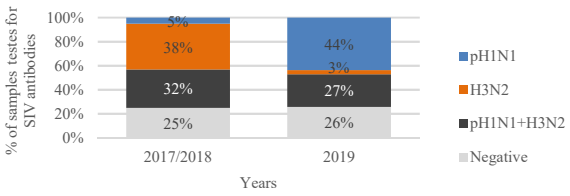

Figure 1. Percentage ratio of SIV subtypes detected in routine samples from 2012 to 2019.

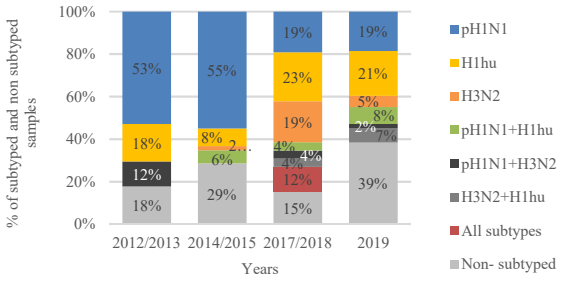

Figure 2. Percentage ratio of serum samples tested for antibodies against pH1N1, H3N2 and pH1N1 plus H3N2 during 2017 to 2019

Discussion and Conclusions

The decrease of pH1N1 occurrence in Brazilian swine herds from 2017, comparing to 2012 to 2015, may be related to vaccine pressure with the beginning of pig vaccination in 2014. Moreover, the change of pH1N1 subtype in human vaccine in 2016 (5) could also contributed for the observation, highlighting both the importance of human to swine transmission route and of monitoring influenza virus circulation in both species. In swine population, it was notable the increase of H3N2 (in 2017/2018) and H1hu (in 2017-2019) occurrence. This evidence is important, since vaccines against pH1N1 do not protect against these subtypes. Continuous surveillance of SIV subtypes, update of vaccines, in pigs and humans, is essential to understand the dynamics of SIV in Brazil.

Acknowledgments: CNPq and FAPEMIG

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Characterization of PEDV circulating strains in Spain between 2014 and 2019 reveals the introduction of a new recombinant PEDV clade

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Introduction

Porcine epidemic diarrhea virus (PEDV) is an Alphacoronavirus with a genomic organization that includes: ORF1a and ORF1b, coding for 16 non-structural proteins; and ORF2 to ORF6, for the spike (S), envelope (E), membrane (M), ORF3 and nucleocapsid (N) proteins, respectively. The virus re-emerged in 2013 (USA and Asia) causing devastating outbreaks mainly associated to non-INDEL strains. In Europe, PEDV outbreaks were sporadically reported before 2014. Since 2014, the virus re-emerged in many countries; however, the strains involved were, with a single exception, low virulence strains (S-INDEL strains). The main objective of this work was to characterize the circulating PEDV strains in Spain after its re-emergence (2014-2019).

Materials and Methods

Thirty field strains collected between 2014 and 2019 from unrelated diarrhea outbreaks in Spain were selected, after the positive identification of PEDV by using a one-step RT-PCR assay, targeting a 651 bp fragment of the S-gene.[1] The whole sequence of 23 PEDV strains were obtained using two approaches: 14 genomes were obtained applying a RNA virus-specific tailor-made NGS protocol;[2] while the remaining nine genomes were obtained by direct amplification and Ion Torrent technology sequencing of eight overlapping fragments. Finally, in seven samples only the sequence of the S-gene was obtained.

The evolutionary relationships among the 30 strains were studied with a ML phylogenetic tree, using the GTR model and 1,000 bootstrap replicates to estimate the confidence of the internal branches of the tree. The presence of recombinant fragments was evaluated with the RDP program.

Results and discussion

All samples clustered within the S-INDEL strains in two branches that follow a temporal pattern: all cases dated prior to 2017 (20) plus one sample from 2018 in one branch, and the remaining nine samples – all dated between 2017 and 2019 – in a second branch. In addition, these nine samples present a recombinant segment spanning 400 bp in the 3' end of the S-gene. The recombination event involved a PEDV strain as a major parental strain, and a *swine enteric coronavirus* (SeCoV) as a minor one (Figure 1).

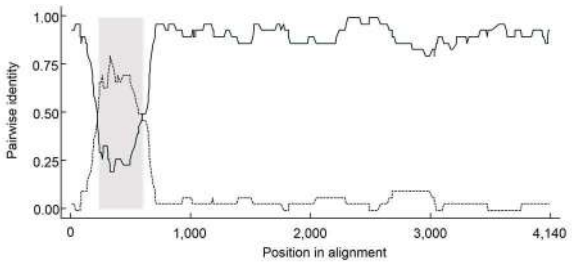

Figure 1. SimPlot graph representing the recombination analysis. The continuous and dashed lines represents the major (PEDV) and minor (SeCoV) parents, respectively, while the recombinant region is marked in grey.

The recombinant event in the 5' end of S-gene with SeCoV reported in these isolates was described previously in Hungary and Slovenia,[3,4] and might provide some advantages, as the S protein is a key target for PEDV neutralizing antibodies.[5]

Conclusions

The PEDV field strains analyzed between 2014 and 2019 in Spain report the substitution of a PEDV clade with a new one bearing a recombinant SeCoV segment in the target region of neutralizing antibodies.

Acknowledgments

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Porcine Parvo Virus new cluster found in the Netherlands

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Introduction

Porcine Parvo Virus (PPV), a single-stranded-DNA virus, has been known for over 50 years and is probably the most important cause of reproductive failure worldwide (1). For years PPV is controlled by vaccination in order to prevent the classical clinical signs of still born piglets and mummies that vary in size and stage of mummification. Apart from this, PPV infections also potentially cause return to estrus and birth of too small litter sizes (1). Within PPV there is evolution (2) and over time in Europe PPV strains are shifting from cluster A to cluster D (3). Recently evidence for the presence of a cluster D strain in the Netherlands was found. This is a summary of the case and consequent findings.

Material and Methods

In a herd of 325 sows in the Netherlands from August 2019 onwards clinical signs of still-born, mummification, embryonal death and infertility (SMEDI) were observed. This resulted in a decrease in the average number of live born piglets per week: over a 9 week period following the start of the problems the number was 81 live born piglets compared to 208 in the 9 weeks before the onset of the problems. Additionally the observed higher mortality of piglets before weaning in the week groups that suffered the SMEDI-problems further reduced the number of weaned piglets in that period.

The PPV product used in this farm was a registered PPV-Erysipelothrix rhusiopathiae vaccine administered according to the vaccination scheme:

- Replacement gilts 2 doses at 26 and 30 weeks of age
- Sows 1 dose every 2nd week of lactation

In the 5th and 6th week after the start of the SMEDI-problems pooled samples of fetuses and mummies were tested by PCR for PCV2, PRRSV and PPV at Merefelt Laboratories (the Netherlands). PPV positive samples were tested by nanopore sequencing at the virology laboratory of Ghent University[4].

Results

PPV was shown in the fetuses and mummies by PCR and by nanopore sequencing. No PRRSV or PCV2 were found. The PPV VP2 region sequence was analyzed and the result (strain 19V196) was compared to available strains in GenBank, showing clustering with recent European strains of cluster D.

Discussion and Conclusion

PPV mutation matters because often used PPV-vaccines are based upon cluster A antigen that do not fully protect against the PPV 27a (cluster D) strain (1, 3, 5). Showing the presence of PPV cluster D in the Netherlands aligns with previous studies that show an increase of PPV cluster D in Europe (3). Recently a new PPV vaccine has come to the market, based upon strain 27a (cluster D) that showed good protection against both cluster A and D strains (6).

We conclude that proving the presence of a PPV cluster D strain in the Netherlands is relevant and may lead to a different choice for PPV vaccine.

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Impact of sows mass vaccination against PCV-2 on piglets prevalence in a Spanish production system

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Introduction

Porcine circovirus 2 (PCV-2), the causative agent for porcine circovirus diseases (PCVD) in growing pigs has also been implicated in reproductive failure of sows[1]. In utero infection of piglets with PCV-2 may serve as a potential source of PCV-2 transmission prior to PCV-2 vaccination. This infection likely makes pigs more susceptible to coinfections with other pathogens and therefore may be associated with PCVD in the growing pig[2]. Piglet vaccination for PCV-2 is routinely used in the pig production globally in weaned piglets. Furthermore, the impact on neonatal prevalence reduction after sow vaccination has been shown in several studies recently. [3,4,5]

The aim of this study was to evaluate the impact of PCV-2 sow vaccination on piglet PCV-2 viremia.

Materials and Methods

The study was conducted in a 900-sow, two sites commercial iberian herd in the center of Spain. In August 2018, reproductive problems appeared at the sow farm: i.e. increase of abortion rate and decrease of fertility rate. At the same time, in weaned piglets appeared an increase of mortality rate.

Subsequently, a cross-sectional sampling was conducted (initial diagnostic), where five aborted sows, and a total of 75 piglets of 1, 3, 6, 9 weeks of age (woa) were bled. PCV-2 and PRRS PCR tests revealed that piglets were positive for both virus since the first week of life. Later on, it was decided to vaccinate and revaccinate sows 4 weeks later with Ingelvac CircoFLEX® (1mL/sow). Piglets were also vaccinated with Ingelvac CircoFLEX® (1mL/piglet) at weaning. With the purpose to have a better understanding of the effect of sow vaccination, the evolution of piglet PCV-2 viremia , and the PCV-2 seroconversion by the ELISA Biocheck test (cut-off = 0,5 S/P), It was sampled sows at farrowing and piglets longitudinally (54 sows and 2 piglets/sow) in 4 different periods depending on the state of gestation of the sows at the moment of the vaccination; prevaccination, sows (Pre-Vac), sows vaccinated between 115-60 days of gestation (115-60 d Gest) , sows vaccinated between 60-0 days of gestation (60-0 d Gest), and sows vaccinated before a new mating (<0 d Gest).

Data was analyzed using Kruskal-Wallis test with Minitab.17.1.0 (2013 Minitab Inc.).

Results

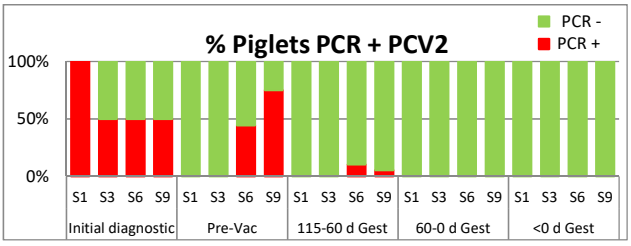

Figure 1: Percentage of piglets PCR PCV-2 positive blood samples at 1,3,6 and 9 woa at different sampling periods, depending on their dam state of the gestation at the vaccination time.

At the beginning of the study, in the Pre-Vac group, a 44% of positive animals with a maximum viral load of 3.1 x 10^6 at 6 woa, and 75% of positive piglets with a maximum viral load of 5 x 10^6 at 9 woa, were still detected (Fig.-1).

In the 115-60 d Gest group, lower values of PCR positive piglets (Fig.-1) and of PCV-2 viral load than the Pre-Vac group were detected, i.e.,11.7% of positive piglets at 6 woa with a maximum viral load of 4x10^4 and 5.88% of positive piglets at 9 woa with a maximum viral load of 2.6 x 10^4. Whereas, in the 60-0 d Gest group and <0 d Gest group, no animals sampled were detected positive to PCV-2 by PCR (Fig.-1).

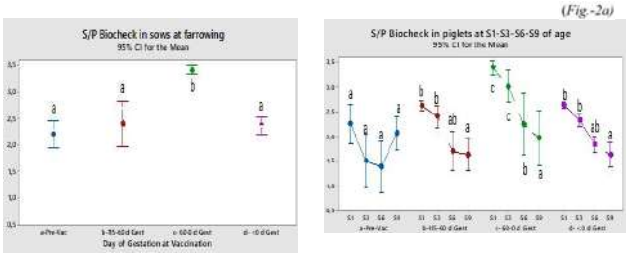

Figure 2: Biocheck PCV-2 Elisa S/P Ratio Test results in sows at farrowing (Fig.-2a), and in piglets at 1,3,6,and 9 weeks of age (Fig.-2b),. Different letters mean significant differences between the different groups in each sampling point (p<0,05).

In the serological analysis, in sows, only the 60-0 d Gest group showed a significant increase (p<0,05) of SP ratios compared with the other groups(Fig.-2a).

On the other hand, in the serological study of piglets (Fig.-2b), all post-vaccination groups, showed greater antibody values, in the first woa, than the Pre-Vac group (p<0,05). In addition, the decay of maternal immunity in piglets issued from vaccinated sows was similar. The only group of piglets that showed an increase in titers at 9 woa was the Pre-Vac group, concomitantly to an increase of the percentage of infected animals, and the PCV-2 viral load.

It was also observed that, viremic piglets were not detected anymore at sampling from the 60-0 d Gest group, and this observation was concomitant with the highest levels of antibodies in these sows and in their offspring at farrowing.

Discussion and Conclusions

According to our PCV-2 PCRs results, we can conclude that the whole-herd-approach concept (defined as sow and piglet disease prevention strategy) has helped to reduce the pre-weaned and weaned piglet's prevalence of PCV-2. Therefore, it looks like the use of CircoFLEX® mass vaccination protocol in sows is a useful tool in order to control PCV-2 prevalence[5].

Further studies are warranted to detect the eventual correlation of this prevalence reduction with an improvement of the production parameters in nurseries and finishing units.

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Swine Acute Diarrhea Syndrome Coronavirus in China

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Introduction

Several pig farms witnessed outbreaks of newborn piglet diarrhea in the winter of 2016/2017 in South China. The mortality rate was 80-90% among piglets less than 1 week old. Pigs tested negative to common swine enteric viruses such as Porcine Epidemic Diarrhea Virus (PEDV), Transmissible Gastro-Enteritis Virus (TGEV), Porcine Rotavirus (PRTV) and Porcine Delta Coronavirus (PDCoV).

Materials and Methods

Diarrheal or intestine samples were collected from 56 farms spread over 15 provinces in China and were shipped in ice to VMRD Beijing lab.

PCR primers for sample analysis were designed based on consensus sequences of *pol* genes (Woo, et al. 2004) and N genes of SADV, PEDV, TGE and PDCoV and the VP6 gene of Group A PTRV.

Nucleic acids were extracted using the Qiagen kits. For coronavirus detection, the conserved *pol* gene primers were used to identify coronavirus positive samples which were further classified by PCR using the N gene based primers. A one-step RT-PCR was done for the PTRV detection. Sequence information obtained from Genbank.

Intestine content of piglets fed with diarrheal samples negative of PEDV, PTRV, PDCoV and TGEV were used for virus isolation. Virus purification was achieved by 3 rounds of plaque purification.

Six days old piglets free of SADV infection and antibodies were used for the pathogenicity assessment. A dose of $1.6x10^6TCID_{50}$ was administered orally to each of 7 piglets. The piglets were monitored for clinical signs for 2 weeks.

Results

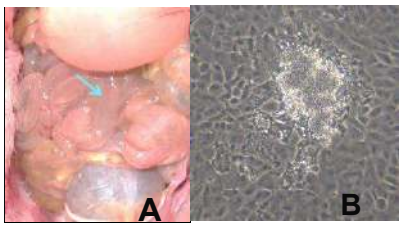
With PCR detection and next generation sequencing, the causative agent of the diarrhea was finally identified as a novel alphacoronavirus, designated as Swine Acute Diarrhea Syndrome Coronavirus (SADSV), also known as Porcine Enteric Alphacorona Virus (PEAV), sharing a very close genetic similarity (≈95%) to the bat coronavirus HKU2. The SADSV was isolated from intestine content of suckling piglets with diarrhea and purified in Vero cells with typical cytopathogenic effects. Pathogenicity study of SADSV showed that five of the seven challenged piglets exhibited medium level of diarrhea and anorexia, but no mortality was observed.

After the first outbreak in 2017 till July 2018, 394 swine diarrhea or small intestine samples were collected from 56 farms in 15 provinces across China including some in the SADSV outbreak region and tested by PCR for SADSV, as well as porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV), porcine rotavirus (PRTV) and porcine delta coronavirus (PDCoV). However, no more SADSV positive samples were identified suggesting that SADSV may not have established itself in pigs. The sample positive rate for PEDV, TGEV, PRTV and PDCoV was 56.3%, 3.0%, 4.8% and 2.5% respectively (Table 1).

Table 1. Summary of Test Results

Enteric virus	PEDV	TGEV	PTRV	SADV	PDCoV
% +ve samples	56.3	3	47.7	0	2.5
% +ve farms	40	3	8	0	4

Figure 1.



A: small intestine from piglets fed with SADV positive sample

B: SADV CPE in Vero cell culture

Conclusions and Discussion

Novel swine enteric alphacoronavirus was identified and purified in China, which shown medium pathogenicity in 6-day old piglets. And the spread of SADSV is limited so far. PEDV remains the main diarrheal viral pathogen in young piglets.

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More control and confidence in the detection of African Swine Fever Virus in difficult sample material

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Introduction

African Swine Fever (ASF) is a threat to animal welfare and one of the most severe diseases affecting wild boars and domestic pigs worldwide. Due to higher risks of the ASF-Virus (ASFV) spreading through contaminated meat, animals or vehicles, accurate detection of the virus is key. The new virotype ASFV 2.0 PCR Kit from INDICAL BIOSCIENCE (formerly QIAGEN Animal Health) enables rapid and reliable identification of ASFV-DNA. The newly developed test kit comes with a double control strategy that offers a higher level of confidence in the interpretation of qPCR results, especially when dealing with difficult sample material.

Materials and Methods

ASFV-DNA was extracted using the silica column-based IndiSpin Pathogen Kit (formerly: QIAamp cador Pathogen Mini Kit) and the magnetic bead-based IndiMag Pathogen Kit (formerly: MagAttract 96 cador Pathogen Kit) from INDICAL. The extracted nucleic acids were then analyzed using INDICAL's new virotype ASFV 2.0 PCR Kit. This triplex qPCR assay contains all the reagents necessary to identify ASFV-DNA, including a positive and a negative control. To exclude the possibility of false negative results, the virotype ASFV 2.0 PCR Kit comes with two internal controls:

- An endogenous internal control (porcine β-actin) ensuring sample presence and quality
- An exogenous internal control added to the lysis buffer, monitoring whether the extraction procedure was successful
- Both controls monitor PCR inhibition as well as the correct PCR setup and cycler program.

The performance of the virotype ASFV 2.0 PCR Kit was tested using in-vitro ASFV-DNA. The validation was conducted on ASFV-positive samples from pigs and wild boars experimentally infected with three different genotypes as well as field samples covering different genotypes. ASFV-negative samples and pig samples positive for other porcine pathogens were also tested.

Results

Testing a titration series of in-vitro ASFV-DNA showed the detection limit of the test kit to be five copies of ASFV-DNA. When testing 245 ASFV-positive samples, the assay demonstrated a high sensitivity. A specificity of 100% was determined by testing 223 ASFV-negative samples. In addition, no cross-reactivity was detected with other porcine viral pathogens such as Classical Swine Fever Virus (CSFV), Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), Swine Influenza Virus (SIV) or Porcine Circovirus 2 (PCV2). Testing the intra-assay and inter-assay variance showed excellent reproducibility and repeatability. Inhibition of qPCR shown by failed or weak control signals and failed endogenous control signals due to sample quality proves the double internal control strategy.

Conclusions and Discussion

The virotype ASFV 2.0 PCR Kit is a highly sensitive and specific solution for detection of ASFV-DNA in samples from pigs and wild boars. The double internal control system provides more confidence in test results, especially when dealing with difficult sample material. In combination with the IndiSpin Pathogen or IndiMag Pathogen extraction kits, INDICAL provides a complete workflow solution for accurate ASFV detection saving precious lab time.

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How are PRRSV-infected fetuses dying?

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Introduction

Pregnant sows infected with porcine reproductive and respiratory syndrome virus (PRRSV2) in the last third of pregnancy can present with fetal death and abortions[1]. We have reported that fetal serum was already positive for PRRSV by 5 days post maternal inoculation (DPI) and that fetal thymus was positive 8 DPI when fetal compromise first appeared[2]. Although gross and histopathologic lesions of infected fetuses have been previously characterized[3,4], we have no knowledge of any study that has investigated apoptosis or hypoxia in fetal tissues. In this study, we investigated gene expression related to cell death (apoptosis) and hypoxia in vital organs of PRRSV2-infected fetuses and compared them to uninfected and control fetuses to elucidate possible causes of fetal death following maternal infection.

Materials and methods

Pregnant gilts were inoculated with PRRSV2 at day 85 of gestation. In weekly batches over seven weeks, 31 inoculated gilts and 7 controls were euthanized at 12 days post infection. Maternal and fetal samples were collected. After PRRSV concentration assessment by RT-qPCR, fetuses were separated in groups based on their preservation status and viral load in placenta, serum and thymus. Phenotypic groups were compared: fetuses from non-inoculated gilts (CTRL, n=30), uninfected fetuses from inoculated gilts (UNINF, n=33), viable high viral load fetuses (HVL-VIA, n=27), and HVL meconium-stained fetuses (HVL-MEC, n=36). Gene expression was assessed by RT-qPCR in fetal brain (BRN, n=48), heart (HRT, n=48) and thymus (THY, n=32). The genes were selected based on their response to infection (IDO1), hypoxia (HIF1a, VEGF, LDHA, NOS2, NOX1) and apoptosis (CASP3, CASP7, CASP8, CASP9, RIPK1, RIPK3). Fold change was calculated for each gene using the $2^{-\Delta\Delta}$Ct method and univariate, non-parametric analysis (Kruskal Wallis followed by Dunn's test) determined group differences within genes (P<0.05 being significant).

Results and discussion

Limited differences were found between UNINF and CTRL fetuses, while HVL and MEC showed a similar pattern of differential expression relative to the CTRL group. The summary of the results for each group per tissue analyzed compared to the CTRL group are presented in Table 1, where black arrows indicate upregulation, white arrows indicate downregulation, and the = sign represent no difference.

Table 1: Pattern of gene expression of the three phenotypic fetal groups for brain (BRN), heart (HRT) and thymus (THY) compared to non-inoculated control fetuses.

	Genes	UNINF			HVL-VIA			HVL-MEC		
		BRN	HRT	THY	BRN	HRT	THY	BRN	HRT	THY
APOPTOSIS	CASP3	=	=	=	=	=	=	=	=	=
	CASP7	=	⇩	=	⇧	⇧	=	⇧	=	=
	CASP8	=	=	=	⇧	⇧	=	⇧	⇧	=
	CASP9	=	=	=	=	⇧	=	=	=	⇩
	RIPK1	=	=	=	=	⇧	=	=	=	=
	RIPK3	=	=	=	⇧	⇧	=	⇧	=	=
HYPOXIA	IDO1	=	=	=	⇧	⇧	⇧	⇧	⇧	=
	HIF1a	=	=	=	⇧	⇧	=	⇧	⇧	=
	VEGF	=	=	=	=	=	=	=	⇧	=
	LDHA	=	=	=	=	=	=	=	=	=
	NOS2	=	=	=	=	⇩	=	=	⇩	=
	NOX1	=	=	=	=	⇧	=	=	⇧	=

Our results indicate that fetal gene expression is altered only after actual fetal infection, with no evidence of hypoxia or apoptosis occurring in the absence of fetal infection. Furthermore, heart is the most affected organ, while brain and thymus appear to be protected at 12 DPI. Upregulation of most genes related to apoptosis indicate that cell death is actively occurring in the fetus, especially in the heart. Besides HIF1a in both HRT and BRN and NOX1 in the HRT, expression of other genes related to hypoxia are not altered at 12 DPI, indicating that the onset of hypoxia might happen in more chronic infection or that it is not an initial cause of fetal death.

Conclusions

There is strong evidence of apoptosis occurring in the heart, and less severely in the brain of PRRSV2-infected fetuses whereas fetal thymus appears to be protected from apoptosis at 12 DPI in spite of being virus positive. There are no strong evidences of fetal hypoxia on these organs at 12 DPI.

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Multiple introductions and reintroductions of the A(H1N1)pdm09-like in intensive pig production in Chile

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Introduction

Influenza A virus (IAV) is widely distributed in the global pig population and it is one of the major concerns for the swine industry, as well as to a constant threat for humans (1). Several genetic variants of A(H1N1)pdm09 linage and various reassortment events between A(H1N1)pdm09 and the typical enzootic subtypes have been reported worldwide in the pig population (2). The A(H1N1)pdm09 lineage, and the reassortant H1N2 and H3N2 lineages with pandemic genes were previously limited reported in Chile (3). Therefore, the objective of this study was to evaluate the introductions of the A(H1N1)pdm09-like in intensive commercial farms of pigs in Chile.

Materials and Methods

Four thousand four hundred twenty-two samples including nasal swabs, oral fluids, and lungs samples were collected from 37 Chilean intensive farms between the years 2013 and 2019. These farms represent >99% of Chilean pig production. The samples were tested by RT-qPCR, viral isolation and full genome sequencing by Illumina Hiseq2000. Finally, phylogenetic analysis was performed using a Bayesian approach. Time-scaled phylogenetic tree reconstruction was performed using the BEAST v1.10.4 package. The Databases were constructed for each of the genetic segments of IAV.

Results

We obtained 142 complete genomes and 241 viral isolates of IAV. The complete genomes were obtained from 19 of the 37 farms sampled. The lineages identified correspond to the A(H1N1)pdm09, H1N2 and H3N2 subtypes. The A(H1N1)pdm09 was identified in all farms we obtained sequences, which in turn co-circulates with the subtypes H1N2 (4 farms) and H3N2 (2 farms). The H1N2 and H3N2 subtypes correspond to reassortant viruses, where all internal genes are A(H1N1)pdm09-like.

Through phylogenetics analyzes, it was possible to identify at least 14 multiple introductions of the gen HA of the A(H1N1)pdm09 lineage (Figure 1) and at least 16 of the gen NA. Also, at least 22 independent introductions of all internal genes were identified. These introductions occurred in the years 2009, 2010, 2011, 2014 and 2016. On the other hand, three different reintroductions were identified in three different farms, in which two of them were only the internal genes and in one of them the complete virus was changed. The changes were from viruses that were introduced in 2009 by viruses of 2016.

On the other hand, recent transmission of viruses between farms were limited and related with gilts movement and the A(H1N1)pdm09 was not involved.

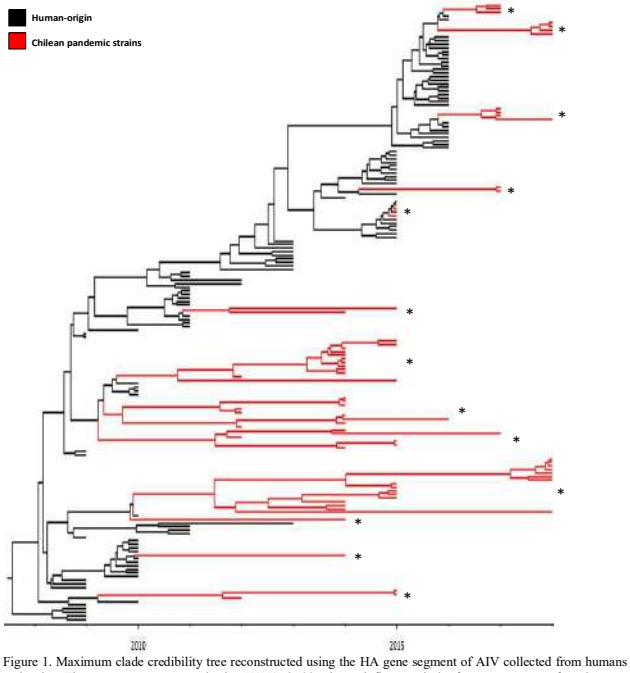

Figure 1. Maximum clade credibility tree reconstructed using the HA gene segment of AIV collected from humans and swine. The tree was reconstructed using H1N1pdm09 subtype influenza viral reference sequences from humans collected from Chile and H1N1pdm09-like subtype Chilean swine viruses. Black branches indicate human-origin strains and red branches indicate Chilean swine strains. (*) indicates different independent introductions.

Conclusions and Discussion

Our results indicate constant introduction of the A(H1N1)pdm09 lineage into Chilean swine. Interesting, more than one introduction per farm was observed in some farms, hence human is a constant source of this virus to swine population. Also, the A(H1N1)pdm09 is able to change other viruses presented in Chilean swine by reassortment.

Our results indicate that the A(H1N1)pdm09 is the mayor IAV concern for Chilean swine industry. The data may be used to improve swine health practices to avoid novel IAV introductions.

Acknowledgments

Fondecyt 11170877, FIV-2017, Beca Doctorado Nacional 2016 Conicyt N°21160963.

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High diversity of intestinal viruses detected using oral fluid metagenomic

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Introduction

Oral fluid is a valuable method for pathogen detection to the most important diseases in swine. In other hand, metagenomic analysis using next generation sequencing becoming an important diagnostic tool for the swine industry. Usually metagenomic has been performed using serum, tissue or secretions focused in affected animals. The objective of this study was identified viral infections performing metagenomic analysis using oral fluids.

Materials and Methods

Oral fluids were collected from pigs at 8-12 weeks old, including 12 intensive swine farms. Farms were PRRS negative and the pigs were not affected clinically by diarrhea. Metagenomic analysis using next generation sequencing were performed to identify the viral ecology using Illumina miseq.

Results and Discussion

Most of the viruses detected in all oral fluid samples were ssRNA, including Porcine Astrovirus (PAstV) 5, PAstV 4, PAstV 2, Mamastrovirus 3, Porcine Sapovirus, Enterovirus, Posavirus, Teschovirus, Kobuvirus and Sapelovirus, which were detected in all farms. Secondly, both dsRNA and ssDNA viruses were detected, including Rotaviruses A, B, C and other Rotaviruses, Porcine Picornavirus (dsRNA), Bocavirus and Porcine like circoviruses (ssDNA) (Figure 1). Less representative were dsDNA viruses which included Porcine herpesviruses and several bacterial phages.

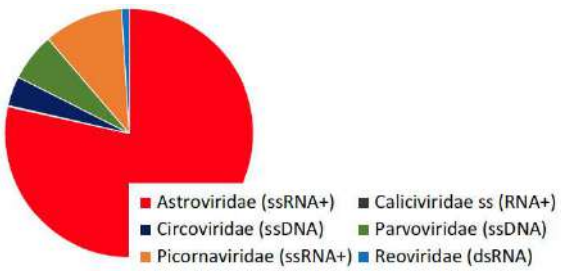

Figure 1. Pie chart showing proportion of viral reads of different families.

Most of the viruses identified were intestinal and non-enveloped viruses, respiratory viruses such as Influenza, Parainfluenza and others previously reported were not observed. Interesting, Porcine Astroviruses were predominant in all farms, human Astroviruses are a major cause of acute diarrhea among children, but the pathogenic role of porcine Astroviruses has been not fulfilled in swine. Recently, Porcine Astrovirus 3 was related with neurologic disease in US but was not detected in this study. In other hand, Rotaviruses are causative agent of diarrhea, but other viruses detected has been recently discovered and its pathogenic role in swine is still unknown.

Conclusion

Metagenomic analysis using oral fluids demonstrated to be useful tool to identify intestinal viruses in swine, especially non-enveloped viruses, but not for respiratory viruses. To improve metagenomic outcome interpretations is necessary compare results using samples from clinically affected and non-affected populations.

Acknowledgments

Fondecyt 11170877, FIV-2017, Beca Doctorado Nacional 2016 Conicyt N°21160963, Beca Postgrado Don Carlos Antonio Lopez .

Using stillborn tongues as an aggregate sample for monitoring PRRSV in an elimination program

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Introduction

Determining Time to Stability (TTS) (Linhares 2014) is a critical step in monitoring the progress of PRRSV elimination from breeding herds. However, the diagnostic process is costly when prevalence decreases below 10% due to sample size requirements. The absence of the PRRSV in newborn piglets is an indicator of stability during these projects. Processing fluids (PF) samples were demonstrated to be useful in the detection of PRRSV in new born piglets using the serosanguinous fluid recovered at the time of castration and tail docking (1,2,). However, in some EU countries the prevalence of castration is very low and regulatory pressures to stop tail docking are increasing. Therefore, alternative sampling methods have been explored including the use of tongues from stillborn piglets (SB). The aim of this study was to evaluate the use of SB tongue fluid (TF) as a sample for the monitoring of a PRRSV elimination program in a 3200-sow farm in Spain.

Materials and Methods

In this study, a previously PRRSV negative 3200 sow closed farrow to wean farm with an internal replacement grow out was used to evaluate this sampling method following a PRRSV outbreak and subsequent elimination project. The infection was confirmed by RT-qPCR and ELISA. The farm started the elimination process after farm-closure and mass infection using the field strain (Time 0). Replacement gilts were kept for 4 months and infected at same time. After time 0, all the piglets were moved off site at the time of weaning. The farm worked in one-week batch farrowing. SB tongues were collected from farrowing rooms starting 12 weeks after time 0. One bag (max. 200 tongues/bag) was taken per room (average 2,5 bags/week), using special bags (BA6141/STR strainer bags, Stomacher®) to recover the serosanguinous fluid. Filled bags were frozen every week (-20C) and sent to the diagnostic lab. Serosanguinous TF from each bag was tested using RT-qPCR.

Results

PRRSV PCR results are summarized in Table 1. [Author Note: At this moment, Jan 31/2020, we are in the 27th week after T0] Threshold values below 35 are considered positive. After 4 consecutive weeks with negative PCR results on TF samples, weaned piglets.
will be monitored using PCR on serum samples for final confirmation of breeding herd stability.

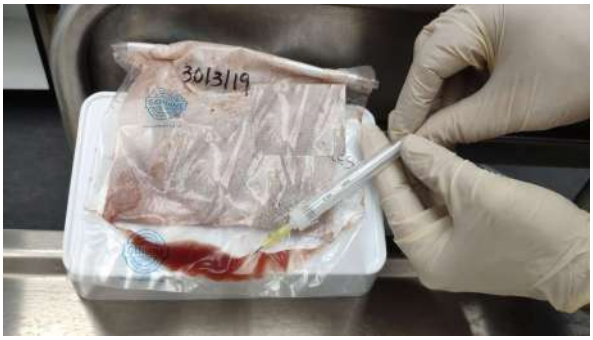

Table 1. PCR results by farrowing room. Ct values above 35, negative

	RTqPCR (Ct values)				
week p.i					
13	18,8				
14	22,6				
15	23,1	23,3	20		
16	27,6				
17	19,7	18,6			
18	25,1				
19	27,5				
20	34	24,7	25,8	27,7	
21					
22	27,9	25,9	29,4		
23	23,9	22,5			
24	26,9				
25	28,6				
26					
27					

Conclusions and Discussion

The study demonstrated the applicability of TF as high-welfare, sensitive, labour-efficient and cost-effective alternative sample for monitoring PRRSV detection in neonatal piglets. Although this is an ongoing project, initial results are promising and the final outcome is expected well before the IPVS program in June 2020.

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Improved finishing pig performance: intervention with Ingelvac PRRSFLEX EU increases carcass weight and reduces mortality

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Introduction

PRRS type 1 infections can play a role in finishing pig performance. However, with many pathogens involved in for instance airway infections it is hard to prove which infection is the limiting factor in optimal pig production. The objective of this study is to evaluate the effect of the PRRS type 1 vaccine Ingelvac PRRSFLEX EU®.

Materials and Methods

In a closed farrow to finish herd, the trial facility VIC Sterksel from the Wageningen University, pigs were historical vaccinated with Ingelvac CircoFLEX and Ingelvac MycoFLEX at the age of 21 days and with Enterisol Ileitis at the age of 6 weeks pigs by the drinking water system. In the first two quarters of 2018 pigs needed to be treated with antibiotics due to bacterial respiratorial and intestinal infections combined with a PRRS infection. From the beginning of June 2018, Ingelvac PRRSFLEX EU was added to the vaccinations on the age of 21 days.

All pigs are identified with an RFID eartag on the first days of life. By coupling date of birth with carcass data, animal performance could be evaluated over time. A historical comparison was made from 2017 until 2019 between the control (CON, n = 1938) and the Ingeval PRRSFLEX EU® vaccinated pigs (PF, n=2465). Only finishing pigs were analyzed (Topigs 20 x Topigs Tempo; full intact boars and gilts). Breeding byproducts were excluded. To compare the effect of PF on mortality, data from the farms management system was analyzed.

Results

Respiratory health, as being judged by the animal care takers, improved resulting in less coughing in the barn and less labor time for treatment and care taking of the animals. Mortality dropped significant from 6.09 (4.14%) pigs/week at an age of 131.6 days (CON) to 4.85 (3.30%) pigs/week at an age of 122.9 days (PF). There was a tendency in lowered variation in mortality week by week (StDev 3.35 CON to 2.61 PF; p=0.051)

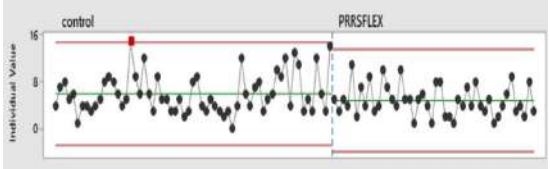

Figure 1 SPC chart of the weekly number of dead finishing pigs. Mortality reduced form an average of 6.09 pigs to 4.85 pigs week (p<0.05) with a tendency of reduced variation (p=0.051).

In total 4,403 finishing pig carcasses were analyzed (Table 1). Carcass weight was 0.7 kg heavier for the PF group (P<0.005; Table 1)

Table 1 Carcass and ADG from birth to slaughter. PF pigs had a significant higher carcass weight (p<0.005).

Group	#	Carcass weight (kg)	ADG Birth-slaughter (gram/day)
CON	1,938	95.66	701
PF	2,465	96.30	704

Antibiotic use decreased to almost zero after the implementation of Ingelvac PRRSFLX EU® vaccination (Table 2). Only individual treatment was needed. Oral treatment of several pigs at the same time was completely ceased.

Table 2 Antibiotic use over the years 2017, 2018 and 2019 in Daily Defined Dosage per animal year (DDDy).

Antibiotic use (DDDy)	Sow	Nursery pigs	Finishing pigs
2017	1.6	7.2	0.6
2018	1.0	3.5	2.4
2019	0.6	1.0	0.3

Conclusions and Discussion

An increase of 0.64 kg meat yield results with an average meat price of €1.50 in an extra benefit of €0.96 per pig. The economic effect of the reduction mortality with 0.83% at an age of 131 days with an ADG of 703 grams equals pigs of 92kg live weight. This reduction in mortality direct loss of value of 80% meat percentage x 92 kg live weight x €1,50/kg meat x 0.83% reduction in mortality = €0.91 per pig vaccinated. The total benefit of vaccination is hereby €1.87 per pig vaccinated which results in a positive ROI.

This study shows that high growth in a conventional health status is possible with only the need for individual treatment.

Detection of porcine reproductive and respiratory syndrome (PRRS) virus for growing pigs in field conditions using tonsil scrapings

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is the most costly swine disease in the United States, affecting pigs of all ages. Sample types commonly used for PRRS virus (PRRSV) detection include serum, which provides high early sensitivity but can be time consuming; and oral fluids, which is convenient and able to detect the virus for longer periods of time[1]. Research on alternative detection methods for PRRSV specifically in growing pig populations is limited[1], and the potential use of tonsil scrapings have not been thoroughly investigated under field conditions.

The objectives of this study were to describe the use of tonsil scrapings (TS) and oral fluids (OF) to detect PRRSV in growing pig herds; and to investigate whether PRRS vaccination status was associated with PRRSV detection in TS.

Materials and Methods

Two PRRS positive farms located in North Carolina were enrolled in this study; a 3,500-head wean-to-finish facility with unvaccinated pigs weaned from a newly positive sow farm (Farm 1) and a 2,800-head finisher farm with pigs vaccinated at processing, between 3-5 days of age (Farm 2). Eight individual TS and 8 pen-level OF samples were collected monthly from each farm as previously described[2,3] using fixed spatial sampling[4], for a period of five months (April to August 2019). Testing was done using RT-PCR (VetMAX® NA and EU PRRS), and a Ct value < 37 was used to declare PRRSV positivity[5]. Statistical analysis was performed in STATA-IC 14. The association between farm vaccination status and TS PRRSV detection was tested using Pearson's chi-squared test, with statistical significance declared at $P < 0.05$.

Results and Discussion

From 64 total TS samples taken over the study period, 57.81% tested positive, and from 64 total OF samples, two (3.12%) tested positive (Table 1). Overall PRRSV prevalence in the unvaccinated and vaccinated herds using tonsil scraping sampling was 50.0% and 70.8% respectively. The two samples that tested positive via OF also tested positive using TS. For both farms, the prevalence of PRRSV positive samples decreased over the 5-month period, except for the last sampling for farm 2 (which yielded 100% positive TS samples), since that was a new group of pigs. All sampled areas set using the fixed spatial sampling approach for both the non-vaccinated and vaccinated farms tested positive at least once on TS throughout the study period. Tonsil scraping samples tested positive up to 21 and 29 weeks post-PRRSV exposure for vaccinated and unvaccinated farms, respectively. Lastly, a Pearson chi-squared test showed no association between farm vaccination status and a positive TS PCR ($P = 0.71$).

Table 1. Proportion of positive detections for PRRSV from RT-PCR results, by month of the study and by farm. Values in brackets represent the total number of samples taken during that month and sample type. Farm 1 refers to the non-vaccinated wean-to-finish facility and Farm 2 refers to the vaccinated finishing facility.

	Farm 1		2	
Month	TS[1]	OF[2]	TS[1]	OF[2]
Apr	87.5% (8)	12.5% (8)	75.0% (8)	12.5% (8)
May	62.5% (8)	0% (8)	83.3 (6)	0% (6)
Jun	50.0% (8)	0% (8)	0% (4)	0% (4)
Jul	25.0% (8)	0% (8)	NA[3]	NA[3]
Aug	25.0% (8)	0% (8)	100.0% (6)	0% (6)

[1]Tonsil scraping samples
[2]Oral fluid samples
[3]Animals were not available for sampling

Conclusion

There are currently no established protocols for PRRSV sampling in growing pig populations. Our results showed tonsil scrapings may be a promising sampling method for PRRSV detection in growing pigs, especially months after the initial PRRSV infection/ vaccination.

Acknowledgments

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An Outbreak of neonatal diarrhea associated to rotavirus type C

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Introduction

Neonatal diarrhea is one of the main causes of preweaning mortality worldwide. Diarrhea is the result of the combination of several factors, including infectious agents, host immunity, environment and management procedures (7). Newborn piglets are susceptible to infection by several enteric microorganisms, including virus, bacteria and protozoans (7). Among viruses, in Argentina porcine rotaviruses (PoRV) groups A, B and C and transmissible gastroenteritis coronavirus (TGEV) has been described associated with subclinical or severely diarrheic outbreaks (4,6). Rotaviruses have a dsRNA genome that contains 11 segments. They are classified into 10 serogroups (A-J) through cross-reactivity of antigens on the VP6 protein that makes up the intermediate layer of the tripe-layer capsid shell of the virus (7). Rotavirus group A, B and C are ubiquitous in swine population; however, group A is the most extensively found on neonatal diarrhea. We perform a multidisciplinary approach in order to clarify an outbreak of a neonatal diarrhea.

Materials and Methods

The outbreak occurred in a multisite farrow-to-finish herd of 7500 sows with AIAO management. Control of neonatal diarrhea included sows vaccination against E. *coli* and feed-back practice in gilts. In August 2018, 2 to 4 days-old piglets exhibited a watery diarrhea affecting 50 % to 60 % litters with 10% to 15 % mortality rates. Diarrhea persisted for 2 weeks. The study involved 30 acute diarrheic fecal samples from piglets less than 3-week-old and pathological studies from 4 euthanized piglets. Segments of ileum and jejunum were fixed in 10 % buffered formalin and stained with H&E. Viral dsRNA was obtained from fecal samples previously diluted ¼ in PBS Tween 0,05 % using a combination of guanidinium thiocyanate and phenol/chloroform nucleic acid extraction methods. All diarrheic fecal samples were tested by 5 % polyacrylamide gel electrophoresis (PAGE) followed by ethidium bromide staining to verify the presence of porcine rotavirus groups A, B, and C (2). Identification of PoRV was conducted using RT-PCR according with the procedure described by Gouvea et al (1). The RT-PCR (PoRVA and PoRVC) products were analyzed using 2 % agarose gel electrophoresis, stained with ethidium bromide, and visualized under UV light. In addition, studies for the detection of TGEV, *E. coli* and *Cystoisospora suis* were carried-out.

Results

Histopathological study showed a mild multifocal villus atrophy in small intestine with low cuboidal to flattened surface epithelium, mild lymphoplasmacytic infiltrate in lamina propria and lymphangiectasia. By PAGE analysis 5 samples (16.6 %) presented a PoRVC electropherotype. The same samples were identified as PoRV group C positive while the remaining 25 samples (83.32 %) were negative. No bacterial or parasitic pathogens were identified.

Discussion and conclusions

PoRV group C has been reported as a cause of diarrhea in piglets under 7 days-old (7) in accordance with our study. A 16.67 % of samples were only positive to group C that was an unexpected finding. Previous studies in Argentina showed only 5 % of PoRV group C prevalence of infection in herds (4). The high rates of morbidity/mortality reported by others suggested the involvement of a mixed PoRV groups or other enteric viruses or bacteria (3). However, in our study, this was ruled-out by complementary studies. Besides, single infections of PoRVC occur more commonly in piglets less than 3 days-old as our reported outbreak (7). Microscopic lesions corresponded with those of a viral infection although, by histopathology, rotaviral from coronaviral infection cannot be distinguished (7). PAGE is the most practical and low-cost method used for PoRV diagnoses; however, it has a low sensitivity. Further, the use RT-PCR assay increased the frequency of detection of PoRV as single or mixed infections (5). The study highlights the importance of PoRV group C alone as a causative agent of neonatal diarrhea.

Acknowledgements

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First detection of porcine circovirus type 3 (PCV3) associated with reproductive disorders in a farm in Argentina

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Introduction

Since 2015 a new circovirus, namely porcine circovirus type 3 (PCV3), suddenly appears within the broad field of emerging swine viruses through metagenomic sequencing studies (4, 5). It belonged from an outbreak of dermatitis and nephropathy-like syndrome (PDNS) in sows associated with reproductive failure -mommies, abortion and stillbirth- (4). Thereafter, PCV3 infection has been involved in cardiac and multisystemic inflammation in mummies as well as in others pigs ages (3, 5).. The PCV3 genome is circular single-stranded DNA with a size of 2000 nt containing two major open reading frames (ORFs), similar as PCV2. ORF 1 encodes the replication-associated protein (Rep) that plays roles in virus replication. ORF2 encodes the capsid (Cap) protein of the virus. PCV3 co-infections with PRRSV, PCV2, TTSuV1, TTSuV2, PPV2, PPV6, and PPV7 has been reported (2). This report describes the etiological, clinical and pathological features of chronic reproductive disorders in a herd in Argentina from which PCV3 and parvovirus type 1 was identified.

Materials and Methods

Since 2016, a 600 farrow-to-finish sow herd located in Santa Fe Province experienced a rise on the average of mommies and stillbirth. Summary of reproductive records were: stillbirth: 6.79%; mommies: 6.13% and abortions: 3.3%. From 2016 to 2019 serum samples from sows with reproductive failure (n: 16) were analyzed for PPV by hemagglutination inhibition test (HIT). Also, pleural transudate of aborted fetus (n: 21) were collected and analyzed by hemagglutination test (HAT) and HIT. Mummified (n=42) and stillborns (n=20) were collected. DNA extractions from pools (lung, heart, liver and kidney) were performed with a commercial kit. Detection of NS1 and VP2 genes of PPV, gD gene of PRV, *cap* gene of PCV-2 and *rep* gene of PCV-3 with specific primers was performed by PCR. From stillbirth 13 hearts were processed for histopathology studies. The *rep*PCV3 gene amplified by PCR was purified, sequenced and the phylogenetic tree was constructed using MEGA program with Kimura 2-parameter model, Maximum Parsimony analysis (MP).

Results

The geometrical medium titters (GMT) of antibodies against PPV in sows sera samples was 2100.96 (512-4096) and the titers in pleural transudate from mummies varies from 1:64 to 1:1024. The HA assay from fetus and mommies was negative. The samples analyzed by PCR not detected any genomic material of ADV and PCV2. Besides, PCR amplification products were observed in 8 pools to PPV and 15 to PCV3. Eight pools were positive for both viruses. In 5 from 13 stillborn hearts studied lesions were located in capillaries and arteriolar located in the subepicardio and myocardio. Lesions were characterized by lymphohistiocytic perivascular infiltration of the blood vessels. Around some vessels and cardiomyocytes connective tissue were observed. The cardiomyocytes were swollen, some of them with coagulative necrosis, myolysis and dystrohic calcification. The phylogram obtained by MP analysis of the partial *rep* gene (985bp) using 25 data sequences showed the grouping in concordance as described by Li G et al. 2018 (2). The Argentine sequence grouped with the strain KX966193 from USA PCV3 classified inside of clade 3b reported by Phan et al. 2016 (4).

Discussion and Conclusions

In summary, antibodies against PPV and DNA genome of PPV and PCV3 were detected in fetuses and stillborn suspected of a co-infection of both viruses as was previously described (2). The heart lesions found were related to PCV3 infection (4). This is the first report of PCV3 infection in Argentina.

Acknowledgments

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Successful PRRSV area control by optimized biosecurity and pig flow management in 40 farms/sites in a highly pig dense area of Denmark within 6 months

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Introduction

PRRSV can be transmitted through aerosols. It travel associated with particles and has been detected in air as far as 9.1 km from swine herds (1,2). Growing pigs represents the absolute majority of PRRSV infected pigs in an area. Growing pigs have a longer duration of viremia and shed PRRSV for a longer period than the adult breeding stock (3) the control of PRRSV circulation in growing pig populations is essential. This can be achieved through a combination of correct biosecurity, pigflow, management and immunization (4).

Materials and Methods

The study, which started January 1. 2019 included 40 farms owned by 15 different producers and involved 10.035 sows from 8 farms, 53.230 nursery pigs placed in 14 different sites and 40.870 finishers placed 25 different sites. All sites and all stages of production, breeding, nursery and finishers were initially sampled to determine the current status following methods described in reference 4. All positive sites were followed by regular testing. A COMBAT (Comprehensive Online management Biosecurity Assessment Tool) survey was conducted in all farms/sites to guide improvement of the biosecurity level, management and pigflow. Depending on PRRS status, pigflow and placement in different sites were adjusted. Some sites were partlially de-populated

Results

First of January 2019 all sow sites except one sow-nursery site were stable positive by Ingelvac PRRS MLV vaccination or negative (following AASV definition (5)) Only strain present in the area was PRRSV2. 4 nursery and 12 finisher sites were exposed to PRRSV. 6 month later, all nursery and finisher sites were PRRSV negative. The one sow-nursery site remained unstable. The progress in serologically PRRS negative sites is illustrated in fig1 and fig. 2.

Conclusions and Discussion

Combination of improved biosecurity, pigflow management and placement order in a large number of herds in a very pig dense area was able to eliminate PRRSV circulation in sow, nursery and finisher sites at the same time as immunization of gilt replacement stock was maintained. This is by far the largest successful PRRS control project in Denmark.

Acknowledgments

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Figure 1. Area map January 2019. Green dots; location of sites without PRRS exposure. Red dots; location of sites, which had been exposed to PRRSV. Yellow dots; vaccinated gilt quarantine and acclimatization.

Figure 2. Area map July 2019.

Comparative Field Efficacy of FLEXcombo® with Inactivated PCV2 Whole Virus Vaccine and Ingelvac MycoFLEX® Combination in a Malaysia Farm

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Introduction

PCV2 vaccine is one of the crucial vaccines in the swine industry. The key aspects for choosing a PCV2 vaccine are efficacy, safety and convenience.[1] Production performance which comprised of average daily gain (ADG) and mortality rate are the easiest way to measure vaccine efficacy.[2] The objectives of this study is to compare PCV2 vaccination efficacy in the field between CircoFLEX® and inactivated whole-virus vaccine C (PCV2a).

Materials and Methods

The trial was conducted in a CSF and PRRS-endemic without PRRS vacccination, farrow to finish farm with 800 sows in Malaysia. 180 three-week old piglets were randomly selected, tagged and assigned into the following groups: Group A-FLEX (n=90) were vaccinated with FLEXcombo® (2ml IM) at left neck and Group B-CV (n=90) were injected with MycoFLEX® on left neck (1ml IM) and whole-virus vaccine C (PCV2a) (0.5ml IM) on right neck. The parameters taken into account were rectal temperature, body weight and mortality rate at each stage. Rectal temperature was taken 3 hours before, 3 hours after and 24 hours after the vaccination. The body weight prior to the vaccination, at weaning, 50-day-old, 80-day-old, 120-day-old were recorded and analysed. The sick pig were moved to the quarantine area and considered as culled pigs according to farm's normal practice. All the piglets were co-mingled together to ensure the same growing conditions with ear-tag to differentiate the groups. The ADG is tested against t-test.

Results and discussion

There was no significant difference (CI 95%) in rectal temperature after vaccination between the two groups of pigs. At day-120, the FLEX group was on average 4.43kg heavier than the CV group which is significantly different ($p<0.01$). In addition, the FLEX group also showed better uniformity than the CV group with the standard deviation of 7.06 as compared to 8.16 for CV group. The pig mortality of the FLEX group outperformed the CV group by 20%. The ADG of pig is significantly favoured by the FLEX group over the CV group ($p<0.01$).

Table 1. Production parameter

	FLEX	CV	Diff.	p-value
Pigs No.	90	90		
Av. BW at each stage (Mean ± SD)				
Starting (kg)	4.12 ± 1.00	4.25 ± 1.13	-0.13	
Weaning (kg)	6.16 ± 1.51	6.09 ± 1.58	0.07	
D50 (kg)	12.19 ± 3.27	12.29 ± 3.62	-0.11	
D80 (kg)	22.25 ± 5.09	21.48 ± 4.43	0.77	
D120 (kg)	47.55 ± 7.06 [a]	43.12 ± 8.16 [b]	-4.43	<0.01
ADG (g/day)	396.25[a]	359.33[b]	36.92	<0.01
Mortality & culling rate (%)	43	63	-20%	

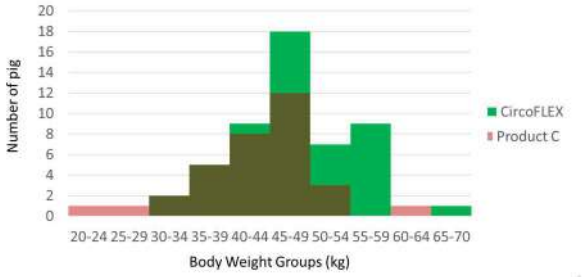

Figure 1. Body weight distribution on D120

Conclusions:

FLEXcombo® was proven to be more efficacious in terms of significantly better ADG and lower mortality rate. Moreover, FLEXcombo® required less labour work and resulted less stress to the pig because only a single injection is required.

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Pathogenic characteristics of the "Russian" group genotype 1 PRRSV-1 strain

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Introduction

The genotype 1 of Porcine reproductive and respiratory syndrome virus (PRRSV) consists of several genetic subtypes. Subtype 1 strains basically are low pathogenic while most of the subtype 3 strains are highly pathogenic, subtype 2 strains are moderately or highly pathogenic. Russian isolates of subtype 1 PRRSV-1 form a separate clade based on genomic phylogenetic analyses. Current Russian strains have typical for subtype 1 ORF7 length, and greater genetic diversity than strains circulated around the world. There is limited information about biological properties of these strains. In 2016 we isolated a subtype 1 PRRSV-1 from lung tissue of dead weaning piglets from the endemic farm in Siberia. The strain was named Tyu16 (Figure 1). In this study we analyzed the genomic structure of this isolate and its performance in experimentally infected naïve piglets.

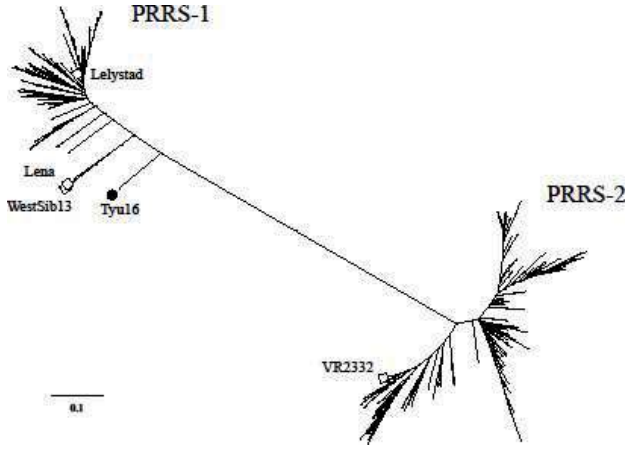

Figure 1. Phylogenetic tree based on complete genomic sequences of 534 PRRSV strains. The Tyu16 genomic identity with the prototype strains of genotype 1 subtypes 1, 2 and 3, (Lelystad, WestSib13 and Lena). is 78.1, 78.1 and 77.7 %, respectively.

Materials and Methods

Porcine alveolar macrophages (PAM) were used for virus isolation and determination of a viral load. Serum samples were tested using a commercial ELISA PRRS 2.0 (INGENASA, Spain) and RT-PCR (VETBIOCHEM, Russia). 1.

Ten three-week-old conventional pigs were randomly divided into two groups of five animals. Animals from the first group were infected with Tyu16 strain of PRRSV-1 (lg 4,33 $TCID_{50}$ per piglet). Animals from the second group were mock inoculated with 5 ml of supernatant of non-infected PAMs. All piglets were euthanized at 21 dpi.

Results

Viremia was detected in all Tyu16-infected animals from 4 to 11 dpi, and 50% of the animals stayed viremic up to 14 dpi. Animals from the non-infected group remained seronegative until the end of the experiment. Pigs inoculated with the Tyu16 isolate seroconverted at 11 dpi. The level of PRRSV-specific antibodies in Tyu16 infected group was growing until the end of the experiment. Mild fever was observed from 4th to 11th dpi in Tyu16 infected animals (Figure 2). Mean temperature in the group was 40,2-40,4°C, with maximum of 41,3°C. All pigs infected with Tyu16 periodically showed mild dyspnea for no more than four study days.

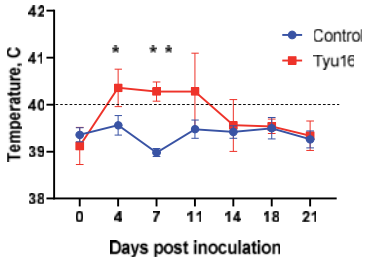

Figure 2. Body temperature upon inoculation with the Tyu16 strain. Asterisks mark statistically significant differences with the control group (**: $p < 0.01$).

Conclusions and Discussion

Continuous circulation of subtype 1 PRRSV-1 in Russia resulted in formation of the new genetic group reflecting growing diversity of PRRSV-1 and their potential recombination with viruses of subtypes 2 and 3. The Tyu16 isolate representing Russian subtype 1 PRRSV-1 group, demonstrated low pathogenicity. It was close by pathogenicity to Western European PRRSV-1 strains.

Acknowledgments

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Implementation of PRRS surveillance using processing fluids (PF) RT-PCR Ct value as predictor for nursery mortality

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Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS) is still one of the most important swine diseases worldwide. Routine PRRS virus surveillance is needed to track progress towards herd stabilization[1]. Disease surveillance is defined as a systematic, ongoing collection and analysis of information related to animal health and the timely dissemination of information to those who need to know for making decisions[2]. Processing fluids (PF) was defined as an aggregated (population) sample, derived from the serosanguinous fluid recovered from piglet castration and tail docking, and described as a new population-based tool to monitor PRRS virus status in processing age piglets[3,4]. The objective of this study was to assess the association between PRRS virus RT-PCR Cycle-Threshold (Ct) values and subsequent mortality at 9 weeks postweaning.

Materials and methods

A 12-month processing fluids-based PRRS virus surveillance program was performed in four breed-to-wean farms with (~3,000 sows each) in a single production system located in south-east Iowa, USA. At each processing day (4-5 days of age), the liquid from all tissues collected was extracted and stored in a 50 mL Falcon Tube (Fisher Scientific, Waltham, MA) and kept refrigerated during a week and sent weekly to the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) where RT-qPCR was performed in pooled samples at each sow farm (1 PCR per week). Three sow farms suffered PRRS reproductive outbreaks during the study, these outbreaks were defined as a combination of presence of clinical signs particularly aborts and off-feed sows and the detection of wild-type PRRS virus in processing fluids or aborted sows. Mortality was recorded at nine weeks post-placement in the nursery from cohort groups (N=173) that had their processing fluids collected and RT-PCR tested. These cohorts were identified using weaning schedule data from the production system, including the four sow farms and 29 nursery sites as downstream flow. Categories based on Ct values distribution were built as low (17-24 Ct, n=20), Medium (24.1-30 Ct, n=53), High (30.1 – 36.9 n=23) and Negative (37 Ct, n=77). A generalized linear mixed model was built to assess the association between mortality percentage at 9 weeks post placement and Ct value at processing, sow farm origin, and season of the year (Summer, Autumn, Winter and Spring). A Tukey pairwise comparisons was run to identify differences between Ct values categories.

Results and discussion

Sow farms included in this study sent 245,624 pigs to 29 nurseries during the follow-up period. Overall mean mortality percentage rate at nine weeks post-placement was 8.3% (95% CI 8.2%-8.4%). Analysis showed a statistically significant association between nursery mortality during the 9 weeks post-weaning period and the PF RT-qPCR Ct values. Mortality in pigs classified as Low Ct category in PF (17-24 Ct values) had the highest mortality. Pigs with Ct values in PF classified as medium and high categories had similar mortality, but pigs in medium category had higher mortality than negative groups (Figure 1).

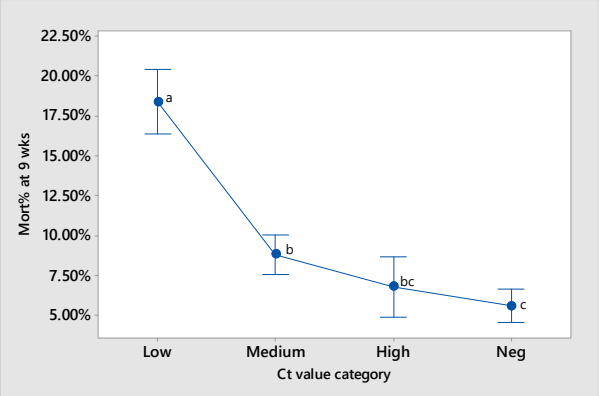

Figure 1. Mortality by Ct values category.
Generalized Linear Mixed Model. Tukey Pairwise Comparisons. Mort. Means that do not share a letter are significantly different (P-value = 0.001).

Conclusion

An association between Ct values in PF and mortality in the nursery was observed, the highest the Ct value, the lower the mortality at 9 weeks in the nursery. These results suggest the possibility of using Ct values in PF as predictor indicators for nursery mortality offering a practical way to establish PRRS surveillance in the sow farm and make decisions about health interventions to anticipate mortality outcomes in the nursery.

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Time to first wild-type PRRS virus detection analysis in vaccinated growing pigs

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is a devastating disease affecting pigs. PRRSV economic impact in growing pigs has been estimated at $4.67 per pig due to its effects on average daily gain, feed conversion, and mortality[1]. Despite the significant cost, we have limited information on the distribution and frequency of PRRSV infections in growing pigs particularly in herds that use vaccination to control the disease. The objective of this analysis was to determine Wild-Type PRRS virus (WT-PRRSV) detection time in vaccinated growing pigs housed in wean to finish sites.

Materials and methods

Sixty-three wean to finish (WTF) sites from 10 production systems located in the Midwestern US were enrolled in an observational prospective cohort study that took place between September 2017 and June 2018. One cohort of pigs per site was enrolled and WTF site selection criteria included: 1) pigs sourced from PRRSV negative or stable breeding herds (based on the AASV PRRSV breeding herd classification[2]), 2) pig placement in all-in/all-out sites located in medium to high density areas, 3) willingness to collect monthly oral fluid samples for PRRSV testing, and 4) willingness to share site level information on production and management practices. Oral fluid samples were collected at each WTF site at approximately 3, 8, 12, 16, 20 and 25weeks post-placement using fixed spatial sampling (8 ropes per site). Oral fluid samples were tested individually by PRRSV RT-PCR. Sequencing (ORF5) was performed on the sample with the lowest RT-PCR cycle threshold (Ct) value identified in a given positive sampling event. PRRSV-ORF5 sequences (n=150) were aligned and classified as wild-type PRRSV using a cut-off of > 2.0% nucleotide difference from reference vaccine virus strains. Three out of the initial 63 enrolled sites were PRRSV positive at first sampling point and were excluded from the study. Fifty-four sites out of the sixty used a PRRSV modified live virus vaccine as part of their protocols with 56% (30 out of 54) of the herds using the vaccines either at placement or within two weeks post-placement into the WTF, and 44% (24 out of 54) using the vaccines before weaning either at processing or around weaning age. A Kaplan-Meier survival analysis was conducted in all vaccinated sites considering first wild-type PRRSV detection and then survival curves were compared between sites received pigs vaccinated prior to weaning and sites with pigs vaccinated after weaning into the WTF site.

Results and discussion

Fifty two percent (95% CI 37, 67) of WTF sites did not have WT-PRRSV detected during the study period. The mean for time to first WT-PRRSV detection in all sites was 20.6 weeks (95% CI 19, 22). When comparing sites vaccinated at the sow farm versus those vaccinated at the WTF, the mean time to detection was 19 weeks for the sites vaccinated in the sow farm compared to 22 weeks for those vaccinated in the WTF, with no statistical differences for survival curves (P-value 0.211) Figure 1.

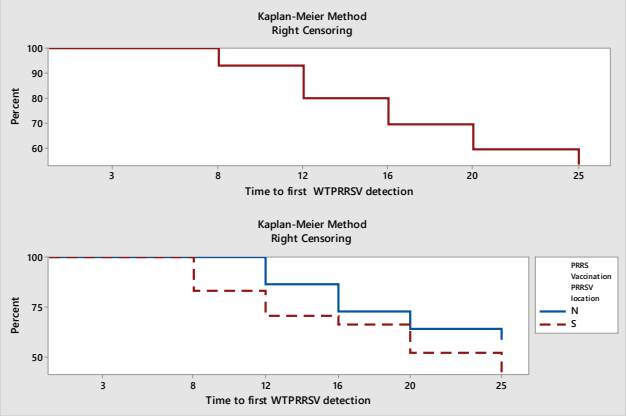

Figure 1. Survival analysis from vaccinated WTF sites.

Conclusion

wild-type PRRSV infections were prevalent in growing pigs (48% of vaccinated sites) originating
from PRRSV stable or negative herds located in medium to high pig dense areas in the Midwest.
Infections were distributed throughout the growing period with WT-PRRSV detection being more frequent towards the end of the growing phase (20 weeks postplacement). Our results also indicate that that timing of PRRSV vaccination does not have an effect on timing of WT-PRRSV detection. Overall, our results highlight the importance of biosecurity in growing pigs. Furthermore, in our study it is likely that detection of wild-type PRRSV in vaccinated herds may have been underestimated due to limitations in diagnostic methods to differentiate vaccine and wild type virus as co-circulation of vaccine and wild-type viruses was a common observation.

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Temporal variation study of the prevalence of Porcine circovirus species and their genotypes (PCV2a, PCV2b, and PCV2d-2) in Brazil from 2009 to 2017

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Introduction

Porcine circoviruses (PCV) belong to the family *Circoviridae*, genus *Circovirus*. Currently, three species are known (PCV1, PCV2, and PCV3), and five PCV2 genotypes have already been described (PCV2a to PCV2e).With the adoption of vaccination for the prevention of Porcine circovirus diseases (PCVD), the concern with the emergence of variants of PCV2 and clinical cases due to vaccine failure became elevated (1,2), since most of the existing commercial vaccines are based on PCV2a. Therefore, the objective of this study was to determine the temporal variation of the prevalence of Porcine circoviruses species and their genotypes in whole blood samples collected in Brazil, which were previously positive for PCV by quantitative PCR (qPCR).

Materials and Methods

Whole blood samples (n=307) were collected from pigs from the main producing regions of Brazil between 2009 and 2017. In the samples tested, age, PCV2 vaccination, farm localization, qPCR data, prevalence, and occurrence of co-infections were analyzed. After DNA extraction by illustra™ blood genomicPrep Mini Spin kit (GE Healthcare), qPCRs were performed with the detection system based on probes (PCV2, PCV2a, PCV2b, and PCV2d-2) or SYBR Green (PCV1 and PCV3). The results of qPCR were reported qualitatively as positive or negative.

Results

From 2009 to 2017, the total number of positive animals found for PCV1, PCV2, PCV2 genotypes, and PCV3 is shown in Figure 1. The prevalence determined for each one in the same period is demonstrated in Figure 2. Concerning vaccination, 57.33% (176/307) of the animals were immunized against PCV2a. For PCV1 positive animals, 50% (17/34) of them were vaccinated, while 56.12% (165/294) of PCV2 positive pigs, and 18.18% (4/22) of PCV3 positive animals were immunized against PCV2a. Regarding co-infections, only 3.91% (12/307) of the samples were positive for PCV1, and 6.51% (20/307) presented co-infection with PCV2. However, co-infection among the PCV2 genotypes was detected in 28.34% (87/307) of the analyzed samples, with the PCV2b and PCV2d-2 genotypes being more observed. Co-infections of PCV3 with PCV2 were found in 6.51% (20/307), PCV3 with PCV1 in 0.33% (1/307), and PCV3, PCV1 with PCV2 in 0.33% (1/307) of the samples tested.

Conclusions and Discussion

The results of this study indicate the highest prevalence of PCV2b, followed by PCV2d-2, PCV1, PCV2a, and PCV3. As previously reported in other countries (3,4), the PCV2d-2 genotype has also been showing a rapid emergence over the years in Brazil (Figure 2). Since the PCV2d-2 may be associated with cases of vaccine failure, more attention should be given to its detection, as well as PCV3 should be considered more carefully. PCV1 and PCV3 were detected with a lower prevalence, but these porcine circoviruses are circulating in pig farms and cases of co-infection occur frequently, as shown in this study. Thus, the results found may be useful for the prevention and control of PCVD in Brazil.

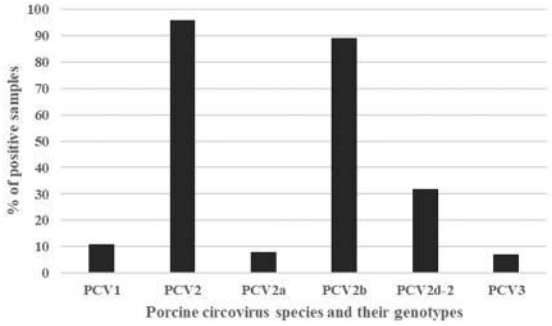

Figure 1. Total positive animals for Porcine circovirus species and their genotypes between 2009 and 2017

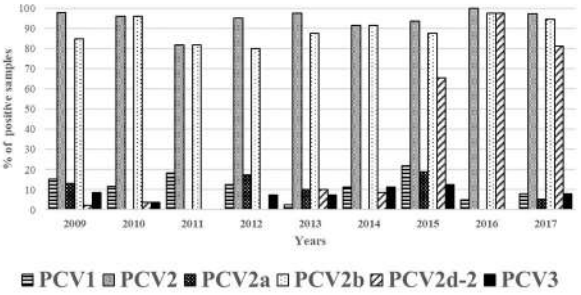

Figure 2. Temporal prevalence of PCV1, PCV2, PCV2 genotypes, and PCV3 in whole blood samples from Brazil between 2009 and 2017

Acknowledgments

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Study of differential gene expression in swine testicle (ST) cells persistently infected with porcine circovirus 2 (PCV2)

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Introduction

Porcine circovirus 2 (PCV2) is considered to be one of the most economically important swine viral pathogens, which belongs to the family *Circoviridae*. The term porcine circovirus diseases (PCVD) is used to designate the clinical syndromes associated with PCV2 (1). As PCV2 infection is characterized by being persistent and causing severe immunosuppression, further research is needed to clarify this important aspect of its pathogenesis. Therefore, the aim of this study was to perform the global analysis of differentially expressed genes (DEGs) between swine testicle (ST) cells persistently infected with PCV2b and control ST cells by RNA sequencing (RNA-seq).

Materials and Methods

DNA and RNA were extracted from ST cells persistently infected with PCV2b (n=2) and control ST cells (n=2). DNA was used to determine the viral load by quantitative PCR (qPCR) for PCV, while the RNA extracted was employed to prepare the mRNA library by SureSelect Strand-Specific RNA Library Prep for Illumina Multiplexed Sequencing kit (Agilent Technologies). The sequencing was conducted on NextSeq 500 System (Illumina). All steps of sequence trimming, mapping with the reference genome (Sscrofa 10.2, Ensembl), and identification of significant DEGs were performed on the CLC bio Genomics Workbench v.7.0.4 program. The Exact Test in the Empirical analysis of DGE tool was used to determine the DEGs significantly, based on a False Discovery Rate (FDR) corrected *P* value <0.05. Significant DGEs with fold change >2 were selected as up-regulated and with fold change <-2 as down-regulated. Then, the gene ontology (GO) and KEEG analyses were carried out by the Blast2GO v.5 program.

Results

The viral loads of the ST cells persistently infected with PCV2b were 1.12 x 10^{11} copies/mL and 5.12 x 10^{10} copies/mL. The ST cell controls were negative for PCV by qPCR. After analyses to identify significant DEGs, 303 up-regulated DEGs and 758 down-regulated DEGs were found by comparing ST cells persistently infected with PCV2b and uninfected cells. According to the GO analysis (level 2), the up- and down-regulated DEGs identified in biological processes are shown in Figure 1. For KEGG analysis, 45 pathways were observed for down-regulated DEGs, whereas for the up-regulated ones 24 pathways were detected. Three immune system pathways were found: T cell receptor signaling pathway, PD-L1 expression and PD-1 checkpoint pathway, and Th1 and Th2 cell differentiation. The DUSP15 up-regulated DEG was verified in the three pathways. The PTPRD, PTPRB, PTPRZ1, and PTPN11 down-regulated DEGs were found in the three pathways, but JAK3 and ZAP70 down-regulated DEGs were identified only in T cell receptor signaling pathway.

Conclusions and Discussion

The PCV2 modulates the host immune system, producing cytokine imbalance, immunosuppression, and disease (2). Since JAK3 is a tyrosine kinase whose role is typically to promote immune response (3), a decrease in its expression can lead to severe immunodeficiency; thus, because JAK3 DEG was down-regulated in ST cells persistently infected with PCV2 compared to control cells, immunosuppression in animals can be induced by decreased JAK3 gene expression. In this study, partial results were presented. Subsequently, the validation of the DEGs is going to be conducted by RT-qPCR and samples of PCV2 positive piglets are going to be tested in order to verify the mRNA expression of these DEGs.

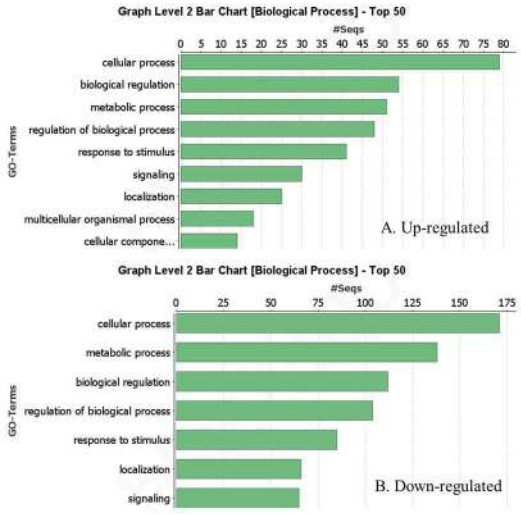

Figure 1. Classification of DEGs (A: up-regulated and B: down-regulated) in biological processes by gene ontology (GO)

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Genetic diversity of porcine circovirus type 2 (PCV2) in vaccinated herds from Brazil

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Introduction

Porcine Circovirus type 2 (PCV2) is considered one of the most relevant pathogens causing economic impact to swine production because of direct losses and subclinical infection. Many genetic studies have proven high heterogeneity of PCV2 because of its ability to recombine and high rate of nucleotide substitution, estimated to be 1.2 x 10^{-3} substitutions per site and year. This substitution rate is the highest recorded for a ssDNA virus and is similar to ssRNA viruses (4,1). Epidemiological studies show an initial predominance in PCV2a during the middle of 1990's followed by a shift in predominance in PCV2b, known as genotype shift (3). A second major genotype shift characterized by the predominance of PCV2d has been reported, indicating that PCV2b has been replaced by PCV2d (previously named mPCV2b) (3). Therefore, the objective of the study was to analyze the genetic diversity of ORF2 gene sequences of PCV2 viruses detected in vaccinated Brazilian farms.

Materials and Methods

A total of 27 clinical samples collected in 2019 and one from 2017, previously tested positive for PCV2 DNA via PCR were used in the study. Samples were collected from animals with PCV2 associated disease (PCVAD) from vaccinated swine herds located in the states of Santa Catarina (SC), Rio Grande do Sul (RS), Paraná (PR), Minas Gerais (MG) and Goiás (GO). DNA extraction was performed using the Promega Wizard® Genomic DNA Purification Kit (Promega, USA). Positive samples were submitted to qPCR for quantification before sequencing (6). The gene ORF2 (capsid) amplification was performed by Nested-PCR using primers previously described (2,6). Sequencing was performed directly from PCV2 DNA amplified by PCR by the Sanger method and nucleotide sequence data analyzed. Consensus sequences were aligned with PCV2 representative sequences from Brazil and reference sequences of PCV2a, PCV2b, PCV2c, PCV2d, PCV1 and PCV3 available in GenBank database (http://www.ncbi.nlm.nih.gov/GenBank). Phylogenetic tree was constructed by the Mega X software, using the Maximum Likelihood method based on the Tamura 3-parameter model and 1,000 bootstrap replicates of the analyzed sequences.

Results

All samples were sequenced with a size of 695 positions in the final dataset. In a total of 27 samples, no PCV2a was found in this study. The PCV2b and PCV2d sequences grouped in separate clusters; 33,3% (9/27) of the samples were classified as PCV2b and 66,7% (18/27) as PCV2d (Figure 1).

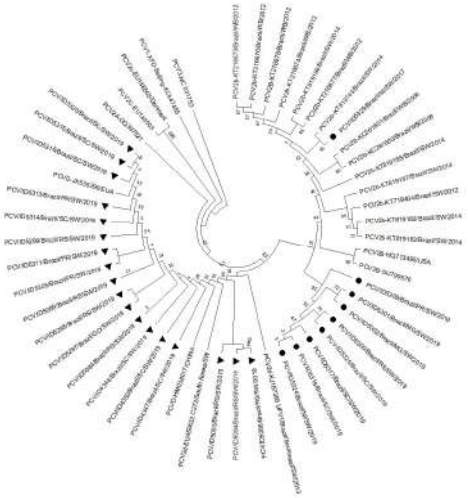

Figure 1. Molecular phylogenetic tree of PCV virus ORF2 gene sequences. The PCV2b analyzed sequences are indicated by solid circle and PCV2d by solid triangle.

All PCV2b sequences from 2019 clustered separately from PCV2b sequences of other years.

Conclusions and Discussion

Studies have shown that PCV2d induces more severe illness (5) and is capable to evade immunity induced by PCVa-based vaccines (7). PCV2d has replaced the previously predominant PCV2b genotype in North America and a similar trend appears to be occurring in Brazil. For the first time a predominance of PCV2d was reported in Brazil.

Acknowledgments

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Efficacy of a high-potency O/PanAsia/OHM/02 FMDV vaccine upon challenge with two heterologous (O/Cathay and O/Mya-98) lineage viruses in pigs

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Introduction

Foot-and-mouth disease (FMD) is endemic widely in Asia and spread of viruses from these regions can cause economic impact. Identifying vaccine strains able to control the spread and outbreaks of FMD is essential for vaccination plans. Vaccination is the most common method of controlling FMD in China. Those programs in China consider the use of oil vaccines containing O and A serotypes either for cattle or pigs (since 2019).

The aim of this trial was the assessment of the cross-protection of a high potency vaccine including the OHM/02 (O/ME-SA/PanAsia) and AKT-III/03 (A/ASIA/AKT/58) vaccine strains against different lineages (O/SEA/Mya-98 and O/CATHAY).

Materials and Methods

Challenge test was conducted in the P3 laboratory at Jinhai's facility in May 2019. Forty pigs at the age of 10 weeks, free of antibodies against FMDV and NSP (non-structural protein) were enrolled. Animals were randomly distributed into four experimental groups (G1 to G4). Each group also included two extra non-vaccinated animals as controls (Ctrl). All vaccinated groups received one dose (2 mL) of a high potency vaccine (Jinhai OHM/02+AKT-III swine vaccine; Batch JH190403, >6 PD50) at 0 days post-vaccination (dpv) by IM route. Pigs from G3-G4 received a booster vaccination at 28 dpv.

Challenge was done following Chinese Pharmacopoeia against O/SEA/Mya98/XJ/2010 at 28 dpv for G1 and 28 dprv for G3; and against O/CATHAY/GX/09-7 at 28 dpv for G2 and 28 dprv for G4. Animals were observed for clinical disease daily for consecutive 10 days, and those who showed lesions in the feet, mouth or snout were considered as non-protected. Each time (28 dpv and 28 dprv) and for each virus strain, 2 non-vaccinated animals were also challenged.

Results

All the pigs in control groups (Ctrl) showed typical clinical disease of FMD after being challenged by the wild type FMDV and were considered infected, granting the trial to be valid. High cross protection rate (90 to 100%) were reached in all vaccinated groups, no matter if they received only one or two vaccinations.

Conclusions and Discussion

The epidemiological situation of FMD serotype O in China is complex due to the co-circulation of four different lineages (O/CATHAY, O/ME-SA/PanAsia, O/SEA/Mya-98 and O/ME-SA/Ind-2001) (He & Li, 2018). High potency FMD vaccines containing divergent topotype/ lineages have been reported to induce effective cross protection (Galdo et al., 2018).

In this study, the challenged animals showed high protection results by both single vaccination and two-shot vaccination.

Hence, vaccinating pigs with high-potency Jinhai FMD vaccine (OHM/02-AKT-III) can successfully be used to confer high cross-protection against serotype O FMDV strains belonging to topotypes/lineages which are circulating in China.

Table 1. Protection rate following one (28 dpv) or two (28 dprv) vaccinations after challenge against heterologous strains (cross protection) in pigs.

	Vaccine strain **O/ME-SA/PanAsia/OHM/02**	
Challenge strain	28 dpv	28 dprv
O/SEA/Mya-98/ XJ/2010	**90%** (G1: 9/10) *0%* *(Ctrl: 0/2)*	**100%** (G3:10/10) *0%* *(Ctrl: 0/2)*
O/CATHAY/ GX/09-7	**100%** (G2: 10/10) *0%* *(Ctrl: 0/2)*	**100%** (G4: 10/10) *0%* *(Ctrl: 0/2)*

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Immunogenicity and cross-reactivity of an O/ME-SA/PanAsia (OHM/02) FMDV vaccine strain against O/SEA/Mya-98 virus in piglets

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Introduction

The spread of foot-and-mouth disease (FMD) virus can have huge economic impact. The epidemiological situation of FMD type O in China is complex due to the co-circulation of four different lineages (1). Vaccination programs are the most implemented method of FMD prevention there, for both cattle and swine. Identifying vaccine strains able to control the spread and outbreaks of FMD is essential for vaccination plans. The aim of this study was to evaluate the antigenic/immunogenic relationship of the O/ME-SA/PanAsia/OHM/02 vaccine strain against a representative strain of one of the main circulating topotype/lineages (O/SEA/Mya-98) in China (1).

Materials and Methods

Trial was conducted in a commercial farm from March to May 2019. Twenty 10-week-old piglets were enrolled after being checked to be negative to antibodies (Abs) against structural and non-structural proteins (NSP) for FMDV. Five animals remained as non-vaccinated controls, while 15 were vaccinated with 2 mL of a high potency vaccine (Jinhai OHM/02 + AKT-III swine; batch JH190403, >6 PD50) by IM route at 0 days post-vaccination (dpv). After 28 dpv a booster vaccination was administered. Serum samples were collected at 26 dpv and 27 days post re-vaccination (dprv). Virus Neutralization Test (VNT) were performed individually to determine the Ab titer against the homologous (OHM/02) and heterologous strain (O/XJ). The cut off was established as 1.5 log10 based on preliminary *in vivo* studies of protection against challenge. The cross reactivity was calculated from the ratio of the mean group VNT titres against homologous and heterologous virus. The interpretation of r1 values follows OIE recommendations (2). VNT data was statistically analyzed by two-way ANOVA (GraphPad Prism V7.0).

Results

All 20 animals resulted negative to structural and NSP when tested by ELISA during the whole study. Control group VNT titer remained non-reactive (below 1.2 log10) at both 26 dpv and 27 dprv (data not shown). Mean VNT titers above the cut off were induced after a single vaccination against the homologous virus. After first vaccination the titers achieved against heterologous virus were above cut off value and slightly lower than against homologous virus (P=0.07). After the booster dose, VNT titres raised significantly and non-statistically differences were detected between homologous and heterologous values. (P<0.001). The r1 values calculated demonstrate the broad cross-reactivity which OHM/02 has against a genetically distant topotype.

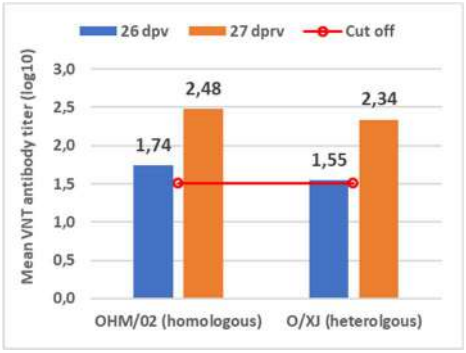

Figure 1. Average VNT titers (log 10) against homologous and heterologous strains.

Table 1. Corresponding **r1 value*** for VNT titers obtained (shown in Graphic 1)

Heterologous strain	Vaccine strain **O/ME-SA/PanAsia/OHM/02**	
	26 dpv	27 dprv
O/SEA/Mya-98/ XJ/2010	**0.65**	**0.74**

*Values greater than 0.3 suggests that the vaccine is most likely to protect against the challenged virus (4)

Conclusions and Discussion

High potency FMD vaccines containing divergent topotype have been reported to induce effective cross protection (3), which coincides with our findings. The vaccine under study confer neutralizing Ab able to react in high titres against O/Mya-98 strain. Cross-protection is related to the virus strains themselves but also the titer of Ab reached, which for the heterlogous strain increased 0.79 log10 after a second vaccination. Thus, vaccinating pigs with high potency Jinhai OHM/02+AKT-III swine vaccine induces high neutralizing titers which confers cross-protection against different type O FMDV strains.

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Immunogenicity evaluation of two commercial Foot-and-Mouth Disease vaccines by serological test in piglets in field conditions at a Chinese Farm

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Introduction

Foot-and-mouth disease (FMD) is highly contagious and considered one of the most important animal diseases and it may cause high economic losses for livestock producers. Vaccination with inactivated vaccines is a routine approach to the control of FMD in many countries [1]. Several companies have been producing FMD vaccine in China and those products contain the antigen of serotype O and A. There are several ways to evaluate the effectiveness of FMD vaccines and the serological test of ELISA is the most convenient one for farms and veterinarians. The object of this trial was to compare two FMD vaccines in the Chinese market by ELISA test to see if there was a difference in the serology for both type O and A.

Materials and Methods

This trial was conducted from June to September, 2019. Three hundred and ninety-seven piglets at the age of 10 weeks, were randomly divided into two groups, whereas 197 were vaccinated with the Jinhai Biotechnology FMD vaccine (OHM/02+AKT-III, swine vaccine; batch JH190403: Group JH) and 200 with vaccine C Jinyu (Re-MYA98/JSCZ/2013 + Re-A/WH/09, swine vaccine, batch JY18392240: Group JY). All animals were injected with the corresponding FMD vaccine at day 0 and re-vaccinated 28 days later, always by IM route. All the animals were raised in the same barn under a similar nutrition and management during the trial. Fifteen serum samples were collected successively in each group at 0 dpv, 28 dpv, 29 days post re-vaccination (29 dprv) and 60 dprv. These samples were labeled and stored until were tested. Solid phase competition ELISA was used in this test (Biao Chi Ze Hui, Beijing, China) for both O and A, separately, following manufacturer instructions. Finally, the Positive Rate (PR) of Group JH and JY was calculated and compared to each other. PR data was statistically analyzed by Chi-square (GraphPad Prism V7.0).

Results

Before vaccination, PR was low and similar among groups for both, O and A (P>0.1). No adverse reaction was observed in any group and after any vaccination. PR against FMD Serotype O in Group JH was significantly higher (P<0.001) as achieved 100% (15/15) positive at 28 dpv while there was only 26.7% (4/15) positive for Group JY at the same time; all samples from Group JH remained positive at 29 dprv and 60 dprv, while PR for Group JY reached 100% at 29 dprv but reduced to 80% at 60 dprv (P>0.1; Figure 1). On the other hand, PR against FMD serotype A in Group JH was 93.3% at 28 dpv while for Group JY was only 26.7% at that time (P<0.01). The seroconversion rate of both groups reached 100% at 29 dprv. At 60 dprv, the PR of antibody against FMD from Group JH was 86.7% whereas the one from Group JY was 66.7% (P>0,1; Figure 2).

Figure 1. Seroconversion as PR by ELISA following vaccination and revaccination for FMD serotype O.

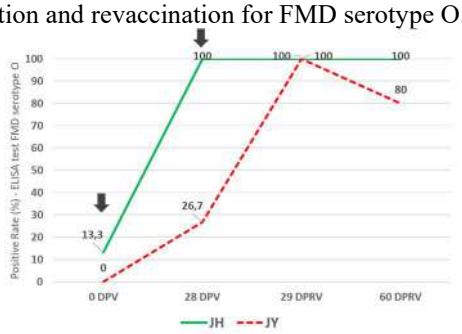
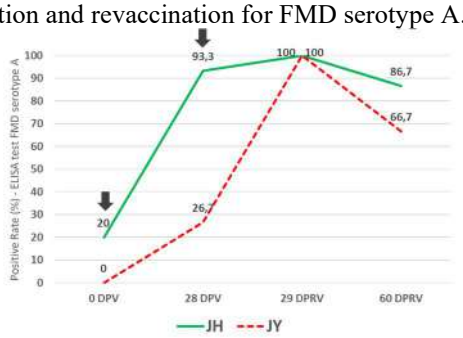


Figure 2. Seroconversion as PR by ELISA following vaccination and revaccination for FMD serotype A.



Arrows = vaccinations. Solid line for Group JH (Jinhai). Dash line for Group JY (Jinyu). Dpv = days post vaccination. Dprv = days post revaccination

Conclusions and Discussion

FMD vaccination is a compulsory program for swine production in China. The recommended vaccination program for piglets is to inject the vaccine twice in one month with the first shot starting around 10 weeks of age. There are several companies who have been producing swine FMD vaccine in China and their products are all bivalent (O and A) inactivated vaccines. In this side-by-side trial, Jinhai's FMD vaccine showed a better performance than the one from Jinyu's, since the seroconversion was faster (onset of immunity) and the positive rate against FMD remained numerically higher for longer time (duration of immunity) for both, serotype O and A.

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Genetic and antigenic diversity of current Porcine parvovirus strains

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Introduction

Porcine parvovirus (PPV) is widespread in swineherds globally and causes reproductive failure manifested by the SMEDI-syndrome. DTU VET receives annually between 50-100 samples from aborted fetuses from Danish herds. During recent years, the average prevalence of positive submissions has increased from 4 % to 17 %. The aim of the study is to investigate the genetic and antigenic diversity of Danish PPV isolates. Furthermore, the cross reactivity of antibodies raised to commercial available PPV vaccines was tested.

Materials and methods

A total of 37 Danish field isolates of PPV were included in the analysis. These isolates originated from diagnostic submissions (fetuses) between 2006 and 2018. DNA was extracted from fetal tissue and the full VP1/VP2 gene sequenced. The sequences were compared to PPV sequences retrieved from GenBank, including available sequences of vaccine strains. Virus neutralization tests were performed to test the level of cross neutralization between antibodies raised to selected PPV vaccines and selected PPV strains.

Results and discussion

The phylogenetic analyses revealed that the sequences grouped into two defined clusters. All of the viruses collected in Denmark 2006-2009, clustered with older strains previously defined as genotype 1. Since 2009, the majority of the Danish field strains clustered together with recent German strains, including the genotype 2 reference strain 27a. Preliminary results of ongoing neutralization tests indicated that there was a marked difference in the level of cross reactivity between PPV isolates belonging to the different phylogenetic clusters.

Conclusion

A significant increase in number of foetuses positive for PPV coincided with a shift in genotype. Results of virus neutralization tests indicated that the genetic clustering also had effect to the level of cross-neutralization and by that indicate that genetic mix-match between PPV field and vaccine strains may influence the efficacy of commercial available vaccines.

Efficacy of three commercial Porcine Parvovirus vaccines in pregnant gilts

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Background & Objectives

The objective of this study was to evaluate the safety and efficacy of a new Porcine Parvovirus Vaccine, named ReproCyc®ParvoFLEX, when administered to gilts before mating. Safety and efficacy were compared to a negative control group and two commercial PPV Vaccines. Furthermore, an ultrasound monitoring of the evolution of the fetal mortality before and after a PPV challenge was also performed.

Materials & Methods

In total 77 gilts were randomly assigned to four study groups. Animals were vaccinated following manufacturers recommendations and were artificially inseminated three weeks after completion of vaccination. Pregnant animals were challenged around 40 days of gestation with a heterologous PPV strain. Foetuses were harvested at around day 90 of gestation and evaluated for the presence of PPV as well as for their condition, size and weight. Ultrasonographic monitoring was performed 4 times during gestation. Parameters such as the number of embryonic vesicles, number of viable and non-viable fetuses were assessed.

Results

All three treatment groups showed statistical differences in comparison to the control group for the: % PPV PCR positive foetuses, % mummified foetuses, mean number of healthy piglets, mean number of abnormal piglets. ReproCyc®ParvoFLEX and Vaccine 2 showed statistically differences in comparison to the control group regarding the "mean number of piglets per gilt" and ReproCyc®ParvoFLEX was the only group showing statistical differences regarding the "% of gilts>11 healthy piglets" parameter and the only that prevented viraemia in the challenged gilts. Furthermore ReproCyc®ParvoFLEX showed statistically better results in the "mean number of piglets/gilt", "mean number of healty piglets/gilt" and "% of gilts>11 healthy piglets" in comparison to Vaccine 1.

No differences were detected between vaccinated groups regarding % of PPV infected fetuses at necropsy, whereas slight differences were detected between groups for body temperature and injection site reactions.

Persistent atypical porcine pestivirus (APPV) infection in gilts

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Introduction

Atypical porcine pestivirus (APPV) is a recently discribed virus that has been associated with Type AII (viral related) congenital tremors (CT) in newborn pigs. APPV was shown to cause CT by direct inoculation of fetal amniotic vesicles with APPV positive serum[1] and intramuscular inoculation of sows during gestation[2]. There is minimal scientific literature addressing the pathogenesis and epidemiology of this virus, including the long-term consequences of CT in gilts. This report characterizes: 1) the duration of viremia and antibody response in gilts born with and in CT positive litters, 2) the transmission of APPV, and 3) if APPV positive gilts either born with CT or born in CT litters give rise to CT/APPV positive piglets.

Materials and Methods

Nine gilts born in CT positive litters were followed from suckling to market weight in a commercial herd and then transported to the National Animal Disease Center for further study. Blood and oral fluids were collected monthly throughout breeding and gestation. The gilts were bred via artificial insemination with APPV negative semen. One gilt was necropsied at the time of breeding. Three groups of naïve contact swine were in fence-line contact with the gilts each for 1 month respectively throughout breeding and gestation to study transmission potential. Blood and oral and rectal swabs were collected at weekly intervals during contact and 1 month after. Post farrowing, piglets were observed for tremors and sampled at farrowing and at 4 weeks-of-age. Rectal swabs, oral swabs, oral fluids and serum were tested for APPV RNA by PCR and the antibody response determined by ELISA. *In situ* hybridization was used to detect APPV in tissues.

Results

Viremia was detected at the majority of time points in the nine gilts for the first five months of age. While viremia was not consistently detected after 5 months, oral fluids and oral swabs were positive for APPV by PCR through farrowing. Interestingly, seroconversion was not observed until around 7 months of age in eight of the nine gilts. APPV was detected by PCR in majority of tissues assayed, Figure 1 illustrates APPV found by *in situ* hybridization in the cerebellum. Transmission of the virus was successful in the first (n=2) and second (n=1) contact groups and all pigs seroconverted. The time to test positive by PCR in at least one sample ranged from 25 to 52 days post contact (dpc). The third contact group (n=2) exposed at the end of gestation had 1 pig that replicated APPV (PCR positive at 35 dpc) and seroconverted, while the other pig stayed negative. Piglets born to persistently infected APPV sows were negative for APPV in serum at birth and did not demonstrate visible tremors.

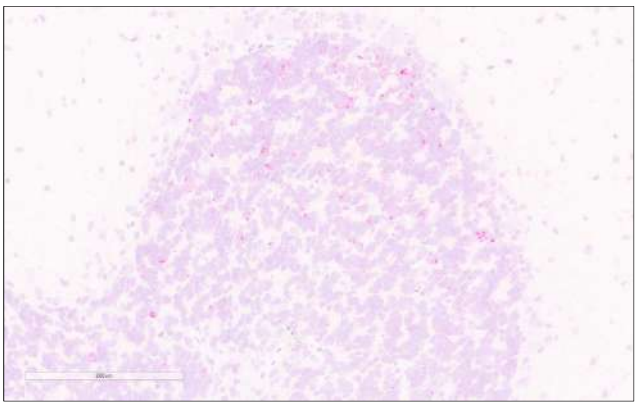

Figure 1. In situ hybridization of APPV in the cerebellum.

Conclusions and Discussion

APPV positive piglets with and without CT tested positive by PCR for APPV for an extended period of time in both serum and oral fluid samples. Seroconversion appeared delayed when considering other porcine viral etiologies, but this may be a result of *in utero* infection during the development of immune tolerance against self-antigens as observed with other pestiviruses. The gilts transmitted virus to naïve contact animals over a 5 month period during breeding and gestation, but did not give rise to APPV/CT positive piglets. Furthermore, the virus was detected by PCR and *in situ* hybridization in a wide variety of tissues and sample types from persistently infected animals. Understanding infection and immune dynamics of APPV and applicable sample types for monitoring infection following exposure can aide control measures for this virus in addition to reducing Type AII CT due to APPV.

Acknowledgments

Animal caretakers for their help with animal sampling. Sarah Anderson and Patricia Federico for their technical assisstance.

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Comparison of historic and contemporary strains of Senecavirus A

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Introduction

In the past, detection of *Senecavirus A* (SVA) from swine samples in the United States was sporadic and originated from cases presenting with various clinical signs. Starting in 2015, there was a significant increase in the detection of SVA in association with farms experiencing vesicular disease[1]. After SVA was proven to be a causative agent for vesicular disease[2], speculation remained surrounding the sudden increase in SVA infections and why attempts to reproduce disease in the past had failed. The hypothesis was that contemporary strains were more pathogenic than historical strains. Our objective was to study disease progression of historical and contemporary SVA isolates in growing pigs and analyze sequence differences.

Materials and Methods

Commercial swine aged 16-20 weeks old were split into 6 challenge groups (n=8). Three historical isolates (SVV001/2002, CAN/2011, HI/2012) and three contemporary isolates (IA/2015, SD/2015, NC/2015) were given intranasally on 0 days post inoculation (dpi) to each of the challenge groups. Animals were regularly bled, rectal swabbed, and oral swabbed through 14 dpi. Animals were observed daily for any clinical signs of vesicular disease. Serum and swabs were tested by real-time PCR for SVA nucleic acid detection. Serum was also tested for neutralizing antibody by virus neutralization (VN) assay. Finally, virus isolated from an animal from each challenge group and sequenced.

Results

All isolates used in the study were able to induce clinical vesicular disease. The number of pigs with clinical signs in each challenge group ranged from 5/8-8/8. Onset of lesions varied from 3-9 dpi. All animals in each challenge group replicated virus and had a least one PCR positive rectal swab for SVA. Most pigs were positive for SVA in oral and/or rectal swabs by 4 dpi and were still positive for virus in rectal swabs at 14 dpi. The exception was pigs challenged with SVV001 where most pigs were negative in rectal swabs by 14 dpi. Cross neutralizing antibody titers against SVV001/2002, HI/2012, and NC/2015 were low for all challenge groups. The group challenged with SVV001/2002 and CAN/2011 had low cross neutralizing titers against all other isolates. The groups challenged with contemporary isolates and HI/2012 had high cross neutralizing antibody responses (Table 1).

The six SVV sequences had a predicted AA identity of 97.5% or greater, and without SVV001, it was 99.2% or greater. Some regions of the proteins were highly conserved (100%) among the 6 isolates such as VP4 and 3B. VP1 and VP3 were 96.2% and 95.8% conserved respectively while, non-structural protein 3A and 2A were both only 88.9% conserved.

Table 1. Cross neutralizing antibody titers. Titer in each cell represent the geometric mean of the group.

	Pig Antiserum					
Virus	IA	SD	NC	HI	CAN	SVV
IA/2015	2048	2048	1024	1722	724	380
SD/2015	2896	1722	1448	1448	609	464
NC/2015	512	431	609	724	152	64
HI/2012	609	609	431	724	181	95
CAN/2011	861	609	1024	1448	1024	210
SVV001	304	215	431	362	304	1024

Conclusions and Discussion

This study demonstrated that vesicular disease can be experimentally reproduced in growing pigs with both historic and contemporary isolates of SVA. In addition, the results suggested there were not large differences in clinical presentation between strains, which was contrary to our hypothesis. Clinical disease observed in SVV001/2002 group was in contrast to other published work where historical isolates did not demonstrated clinical disease[1,2]. As expected, higher neutralizing antibody titers were observed against homologous and more closely related strains. Further research will be needed to help determine the cause of the sudden increase in vesicular disease due to SVA infection in the United States swine population.

Acknowledgments

Animal caretakers for their assistence with animal sampling. Deb Adolphson and Sarah Anderson for their technical expertise. Iowa State University, Kansas State University, Boerhinger Ingelheim Vetmedica, and the National Veterinary Services Laboratories for their contributions of viruses.

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Diversity of RNA viruses in Spanish pig farms with diarrhoea using next-generation sequencing

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Introduction

Diarrhoea is a major cause of economic losses in pig production and RNA viruses such as *porcine epidemic diarrhoea virus* (*PEDV*), *transmissible gastroenteritis virus* (*TGEV*) or *Rotavirus* are among the main etiological agents [1,2]. Furthermore, other RNA viruses including *Kobuvirus*, *Astrovirus*, *Sapovirus* or *Torovirus*, have been detected in pig faeces although their role as causative agents of diarrhea in swine has not been so far fully elucidated [3].

Next Generation Sequencing (NGS) offers a unique opportunity for non-targeted identification together with in-depth characterization of any virus present in the sample. The aim of the present study was to explore the diversity of RNA viruses in faeces of pigs of different ages suffering from diarrhoea using NGS technology [4].

Materials and Methods

The study was performed in 28 Spanish swine farms with diarrhoea outbreaks during 2014-2019 in which a viral aetiology was suspected and occurring during suckling (n=4), postweaning (n=5) and fattening periods (n=19).

Total RNA was extracted from one pooled sample per farm by means of the Trizol LS reagent, subjected to quality assessment (A260 and A280) and submitted for library preparation and deep sequencing in an Illumina Miseq Platform. Reads yielding a QC score >20 were accepted for further filtering. Reads were taxonomically classified with Kraken, mapped against the complete genomes of the RNA virus identified and a consensus sequence for every virus was assembled using a tailor-made script [3].

Results and discussion

Eleven genera of RNA viruses were identified including *Coronavirus* (PEDV in 22 farms), *Rotavirus* types *A* (9), *B* (3), *C* (3) and *H* (5), *Astrovirus* (23), *Kobuvirus* (16), *Pasivirus* (3), *Posavirus* (1), *Sapovirus* (4), *Sapelovirus* (12), *Torovirus* (8) and *Enterovirus* (*porcine enterovirus 15* (1) and *G* (16)). Neither *TGEV*, *porcine deltacoronavirus* nor the recently described *swine acute diarrhoea syndrome coronavirus* were detected. Prevalence of positive farms is shown in Table 1. Interestingly, many farms were co-infected by different viruses (up to fifteen). A mean number of 6 different viruses per sample were identified among suckling piglets, 9 among postweaning and 4.7 among fattener outbreaks.

Table 1: Distribution of positive farms (%) to each investigated virus among neonatal (Neo), postweaning (PW) and fattening (Fat) diarrhea outbreaks.

Percentage positive farms							
Virus	Neo	Pw	Fat	Virus	Neo	Pw	Fat
PEDV	75	100	74	*PasiV*	25	-	11
RVA	50	40	26	*PosaV*	-	20	-
RVB	-	40	5	*SapeloV*	50	60	37
RVC	25	20	5	*SapoV*	-	60	5
RVH	-	20	21	*ToroV*	25	40	26
AstroV	100	80	68	*EntVG*	50	80	53
KobuV	100	100	37	*EntV15*	-	-	5

To note, *porcine enterovirus 15* was detected for the first time in domestic pigs since there is only one sequence recovered from wild boar available up to now in GenBank. Moreover, we also detected *Rotavirus H* and to the best of our knowledge, this is the first description of this rotavirus in pigs in Europe.

Conclusions

This research brings some light into the complexity of the intestinal virome in swine diarrhoea outbreaks. *PEDV* is confirmed as an important etiological agent being the only enteric coronavirus detected. Our results demonstrate that enteric viruses are very common on swine farms and emphasize the need for a better understanding of the role of the different agents involved in pig diarrhoea.

Acknowledgments

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PRRS MLV vaccination of piglets reduces antibiotic use in fattening pigs

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Introduction

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is the causative agent of a disease that affects pigs worldwide and produces large economic losses to the swine industry[1]. Next to direct clinical disease with huge economic consequences, PRRSV affected pigs are also more susceptible to secondary bacterial infections during growing and fattening phases. Therefore, piglet immunization against PRRS with modified live virus (MLV) vaccines is being increasingly used in commercial farms. It has been clearly documented that vaccination of piglets improves the clinical and production results in farms[2,3], but the influence of PRRS vaccination in piglets on the antimicrobial use in fattening pigs has only scarcely been described.

The objective of this study was to assess the impact of early PRRS piglet vaccination on the use of antibiotic treatments during the fattening period.

Materials and Methods

A 700-sow herd with PIC sows started PRRS piglet vaccination between 2-3 days of age (D) in February 2018 with Suvaxyn® PRRS MLV (Zoetis). Pigs from the breeding farm were moved to different fattening farms from an integrated production system, where the use of antibiotic (AB) was monitored and then analyzed in batches of fattening pigs in two stages: 1) before PRRS vaccination (AUG 2015- JUN 2018) and 2) after the start of PRRS vaccination (JUL 2018 – DEC 2019). During the observation period, the genetics of the boar line changed in SEP 2017 from Belgian Pietrain (BP) to PIC boars (PB); along with that change the use of Improvac® was discontinued. The influence of PRRS vaccination on AB use was also analyzed between these 2 periods.

For each batch, antibiotic use during fattening period was registered and expressed as TD100 (number of days a pig receives an antibiotic treatment per 100 days present in the fattening unit = average % time the pigs are treated with antibiotic)[4]. The farms for which results were available before (n=22) and after (n=38) the implementation of the PRRS vaccination were selected from all the available batches and compared by independent samples t-test. (SPSS 25.0).

Results

In total, data of antibiotic use were available for 135 batches of pigs (51,854 pigs, Mean 381 pig/batch (min 90 – max 720). Overall, the TD100 for all these batches was 3.53. After selection of farms where data were available before and after the implementation of PRRS vaccination, 60 batches from 7 different fattening farms were selected for analysis. These batches were marketed between MAY 2016 and DEC 2019. 22 batches were from PRRS not vaccinated and 38 batches were from vaccinated piglets. On average, the TD 100 was 3.61 for these 60 batches. Furthermore, when TD 100 was compared between non-vaccinated and vaccinated batches, the Median was 5.62 vs 2.45 for vaccinated batches (p=0.016) (figure 1). Based on genetics, the difference between BP (average TD100 = 5.49) and PB (average TD100 = 3.24) was not statistically significant (p=0.20).

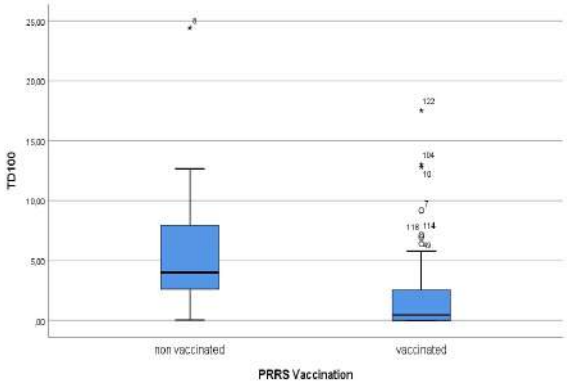

Figure 1. Boxplot of TD100 of batches vaccinated and non-vaccinated with Suvaxyn PRRS MLV.

Conclusions and Discussion

It has been frequently described that production parameters and clinical parameters are improved by PRRS vaccination. In the current field observations, there was a clear reduction of anitbiotic treatements by 54% after the implementation of early PRRS vaccination in piglets with Suvaxyn® PRRS MLV.

This may be explained by a beter control of PRRS virus infection and a consequently lower susceptibility for secondary bacterial infections that often cause clinical disease in fattening pigs. The results of the current study indicate that the implementation of PRRS vaccination in PRRSV infected production systems may help improving the overall health of fattening pigs, and as a result, reducing the need for antiobiotics in pigs contributing to more responsible use.

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Prevalence of PCV2 subtypes in the Benelux

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Introduction

The genome of Porcine Circovirus (PCV2) is changing continuously with the emergence of new subtypes like PCV2b, PCV2d, PCV2e and PCV2f, and further development is ongoing[2]. Genotype changes cause evolutions in capsid structure, affecting the binding-capacity of the virus to the host cells[3]. Within genotype PCV2 , subtypes a, b and d are known for their clinical importance[4]. Currently, recent information on the prevalence of the different subtypes in Benelux is lacking. To generate an overview the prevalence of these 3 different PCV2 subypes within the Benelux was evaluated.

Materials and Methods

Pigs of 61 farms were sampled using Oral Fluids, of which 31 in Belgium and 30 in the Netherlands. Farms were randomly selected by herd veterinarians, and no criteria where included. Animals of around 12 – 17 and 22 weeks of age were sampled on one day with 5 ropes per farm. BE samples were analyzed at DGZ-Flanders and NL samples were analyzed at GD Animal health by Q-PCR for PCV2. 40 farms were vaccinating for PCV2 (V), 20 farms were not vaccinating (NV) and 1 farm had no information about the vaccination. Samples with the highest Q-PCR values per farm were send to the lab of the University of Munich (Laboratory Department for Veterinary Sciences, LMU Munich) for ORF2 genotyping .

Results

In total, 46 out of the 61 farms were positive for PCV2 in Q-PCR in at least 1/5 samples. None of the unvaccinated farms was negative (Table. 1)

Table 1. Positive and negative V and NV farms in NL en BE

		Positive		Negative	
Country	**No. Farms (N)**	**V**	**NV**	**V**	**NV**
NL	30	12	4	14	0
Be	31	14	16	1	0
Total	61	26	20	15	0

In 2 of the 46 positive farms it was not possible to perform the subtyping PCR. Subype a was the predominant subtype 75% (33 out 44 farms) either alone, or in the presence of other subtypes. In 30% (13 out of 44) of the farms 2 or 3 subtypes were present. (Fig. 1) . In vaccinated farms (n=24), and where subtyping was succesfull, there were more than 1 subtype identified representing 21% of the cases.

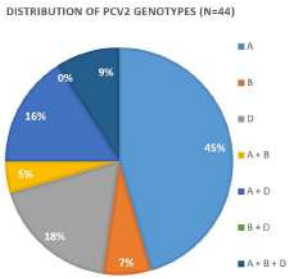

Figure 1. Distribution of PCV2 subtypes in Benelux.

In the other hand, in unvaccinated (n=20) farms, this was 40% of the cases.(Fig. 2)

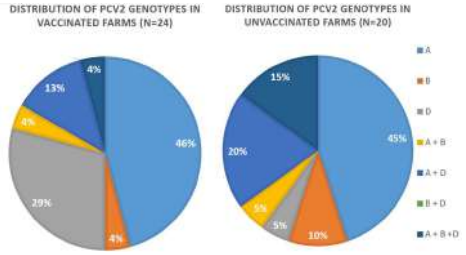

Figure 2. Distribution of PCV2 subtypes in vaccinated and unvaccinated farms.

The geographical region where these farms are located goes from Northern part of the Netherlands to south-east part, and from western part of Flanders to the eastern border in Belgium. There are no indictions for regional differences whether or not 1 or more subtypes of PCV2 occur in the farms at the same moment.

Conclusions and Discussion

This study shows a prevalence of 76% for PCV2 in Benelux. PCV2a appears to be the most common subtype (75%), followed by PCV2d (43%) and PCV2b (21%) respectively. 30% of the sampled farms were positive for 2 or 3 subtypes at the same time: this applies to 21% of the vaccinated farms, versus 40% of de non-vaccinated farms. Based on this preliminary results, it might be possible that vaccination is limiting the number of PCV2 genotypes in a farm.

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Validation of a FMDV 3ABC indirect ELISA for swine oral fluid specimens

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Introduction

Foot-and-mouth disease virus (FMDV) remains uncontrolled in most of the world, with circulation of multiple serotypes in endemic areas (1). Actually, North America is among the few "FMDV-free without vaccination" areas of the world. The current massive level of global trade and traffic means that FMDV anywhere in the world presents a credible risk to U.S. agriculture. In the event of an FMDV outbreak in North America, effective control and elimination will require rapid detection. In turn, rapid detection will rely on an (1) efficient surveillance sampling technology and (2) immediate access to accurate diagnostic assays. Therefore, the objective of this project was to create a FMD 3ABC antibody indirect ELISA for use with swine oral fluids.

Materials and Methods

Prototype serum and oral fluid FMDV 3ABC ELISAs were developed using samples from animals of precisely known FMDV antibody status. In brief, a recombinant FMDV 3ABC protein was produced and used as antigen in the FMDV 3ABC IgG indirect ELISAs. The response characteristics of prototype serum and oral fluid FMDV IgG 3ABC ELISAs were optimized using samples from 3B vaccinated animals. Diagnostic sensitivity and specificity of the ELISAs were evaluated using serum (n = 1,026) and oral fluid (n = 351) samples collected from pigs inoculated with FMDV (serotype O, A, SAT2, and Asia1). Serum and oral fluid samples collected from pigs inoculated with vesicular disease viruses (vesicular stomatitis virus and vesicular disease virus) were tested with the FMDV 3ABC indirect ELISAs to evaluate analytical specificity of the tests.

Results and discussion

The FMDV 3ABC indirect ELISA tests detected specific antibody in serum and oral fluid samples from FMDV-infected or FMDV-vaccinated pigs by 7-14 days post exposure (Figure 1) with no cross-reactivity against vesicular stomatitis virus and vesicular disease virus antibodies.

Thus, the results of this study demonstrated that FMDV antibody in oral fluid and serum and could be detected using an indirect FMDV 3ABC ELISA. Importantly, (1) the response is not serotype specific, i.e., antibody was detected in animals exposed to serotypes O, A, SAT2, and Asia 1 and (2) the assay detects antibody against a non-structural protein which is not present in FMDV inactivated vaccines, i.e., the test provides for differentiation of vaccinated vs infected animals (DIVA).

Conclusions

Diagnostic testing of swine oral fluid samples has proven to be an effective and reliable method for the surveillance of endemic infectious diseases. Implement of a DIVA strategy in infected animals is used to monitor the ongoing success of FMDV eradication and to maintain “FMD-free with vaccination” status in some areas (2). Expanding this methodology will help provide FMDV-infected countries a new tool to control the infection and prepare the U.S. industry for a "worst-case" scenario.

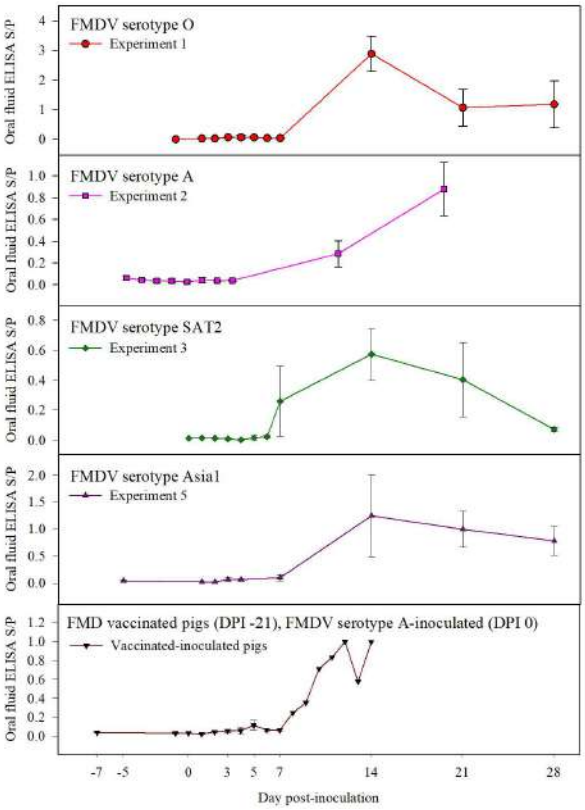

Figure 1. Oral fluid antibody responses on the FMD 3ABC indirect ELISA.

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Histopathological lesions of a novel strain of influenza virus (H1N2) isolate from Chile

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Introduction

Currently in a swine population there are various strains of influenza virus (IAV) such as the classic, H1N1, H3N2 y H1N2 that has economic and public health relevance, being considered ubiquitous in the swine industry worldwide (1). In Chile, novel IAVs-S of the H1 and N2 subtypes have been recently identified in commercial swine farms, which are genetically divergent from IAVs described in other countries (3). IAV produces an acute respiratory disease with a high morbidity. It normally infects the respiratory epithelium producing clinical signs such as cough, fever, lethargy, lack of appetite and anorexia. Grossly, the lesions observed are characterized by having a multifocal pattern and a well-defined multifocal violet red color in the ventral cranial areas of the pulmonary lobes. It is known as chess board lung (2).
It is relevance to know the lesions causes by this novel virus and how this could affect pigs.

Materials and Methods

In order to do this study, 12 pigs, 60 days old, were intranasally challenge with 1 cc per each nostril with the strain A/Sw/VN1401-274/2013 H1N2 at $1x10^6$ in an experimental unit from the University of Concepcion. The animals were sedated and anesthetized, in order to the necropsy at 5, 9 y 15 days post challenge (dpi). Four animals were necropsied each time and grossly lung lesion were recorded and sampled were collected in order to do histopathology and Immunohistochemistry (IHQ). Histopathological lesions were classified in mild (+), moderated (++) and intensive (+++). Additionally, bronchial submucosa infiltration of inflammatory cell (BSII) were registered, as well as peribranchial infiltration (PBI), peribronchiolar infiltration (PI), the level of collapse of pulmonary lobules (CPL) and the level of interstitial infiltration of the pulmonary parenchyma (IIPP) were analyzed.
The viral lesions were confirmed by immunohistochemistry.

Results

Grossly lung lesions showed congestion and edema, collapse of the pulmonary lobules with a chess board scheme (table 1). Additionally, enlarge and hemorrhaged lymph nodes were observed. Histopathological analysis of the lungs from challenged animals showed different types of inflammation in this organ, in addition to the pulmonary collapse (table 2).

Table 1. Grossly lesions associated to a challenged with a novel H1N2 IAV. C E: congestion and edema, EHLN: enlarge and hemorrhaged regional lymph nodes, TS: Tracheal secretion and CL: collapse of lobules.

Lesion grade	C E	EHLN	TS	C L
Without lesions	41,6%	50,0%	50,0%	0,0%
Mild	8,3%	0,0%	16,6%	50,0%
Moderate	50,0%	50,0%	33,0%	8,3%
Intensive	0,0%	0,0%	0,0%	41,6%

Table 2. Microscopically lesions associated to a challenged with a novel H1N2 IAV. BSII: bronchial submucosa infiltration of inflammatory cells, PBI: peribranchial infiltration, PI: peribronchiolar infiltration, CPL: collapse of pulmonary lobules, IIPP: interstitial infiltration of the pulmonary parenchyma.

Lesion grade	BSII	PBI	PI	CPL	IIPP
Without lesion	0,0%	8,3%	0,0%	41,7%	0,0%
Mild	25,0%	41,7%	25,0%	33,3%	0,0%
Moderated	41,7%	41,7%	75,0%	25,0%	25,0%
Intensive	8,3%	8,3%	0,0%	0,0%	75,0%

Conclusions and Discussion

In the present study we were able to reproduce the typical lesion of IAV with a novel strain, where the more importance grossly lesions were moderate CE and EHLN, and mild or intensive CL. The more important microscopical lesions were a moderated BSII, PBI, and PI, and an intensive IIPP.

Acknowledgments

Department of virology of the University of Chile.

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Viral dynamic of a novel IAV H1N2 strain in pigs

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Introduction

Influenza A virus (IAV) has economic and public health relevance, being considered ubiquitous in the swine industry worldwide. In Chile, a novel H1N2 IAVs high prevalent lineage has been recently identified in commercial swine farms, which are genetically divergent from IAVs described in other countries (1). The viral excretion of this virus has been previously evaluated in guinea pigs (3) but its viral dynamic has been not evaluated in swine.

The objective of this study was to determine the viral excretion of this high prevalent novel H1N2 IAV in pigs.

Materials and Methods

Thirty-two 60 days-old pigs were intranasally challenge with the strain A/Sw/VN1401-274/2013 H1N2 at $1x10^6$ in an experimental unit from the University of Concepcion.

To determine the viral excretion, nasal swabs were collected at 3, 5, 8, 10, 13, 15 and 22 days post inoculation (dpi). The samples were collected using rayon swabs and preserved in Stuart medium.

Additionally, to identify viral load in the lungs, 4 animals were euthanized at 5, 10 and 15 dpi, collecting deep tracheal samples. After collection, samples were preserved at -20 C.

Samples were tested by real-time PCR and further titrated in MDCK cells. Cut value to determine a positive samples was Ct 35. The infectious dose for 50% of cultured tissues ($TCID_{50}$) was calculated according to Reed and Muench method (2). The virology assays were performed at the virology laboratory at the University of Chile.

Results

All pigs were successfully infected by the inoculation, confirming that the novel H1N2 is indeed pathogenic for swine. Overall PCR results are illustrated in Figure 1. The virus was detected in all pigs until 5 dpi, the viral clearance starting at 8 dpi and not detected IAV was observed at 13 dpi of later.

Viral titers were in concordance of real time PCR results. However, only at 3 and 5 dpi $TCID_{50}$ was observed. 23 out of 32 present viable virus at 3 dpi and 26 out of 32 at 5 dpi. Not viable IAV was identified at day 8 or later, which can be explained for the lower Ct values, but also due to the samples were not preserved in viral transport media.

On the other hand, real-time PCR to the tracheal swabs determine 100% of positive samples at 5, 10 and 15 dpi but viable virus was obtained only at 5dpi.

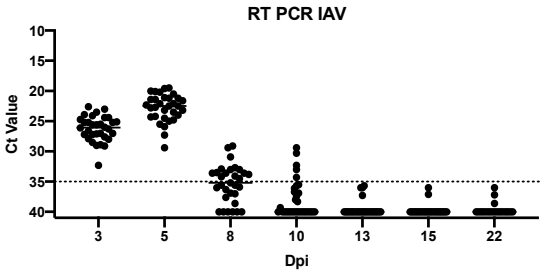

Figure 1. RT PCR results of nasal swabs. Positive samples are above the Ct 35.

Conclusions and Discussion

The results evidence the viral excretion of the novel H1N2 IAV, which was similar to other IAV isolates. The virus is excreted for 10 or less days, and the viral clearance starting at 5 dpi, which is important for the diagnostic.

These results will help to better understand the viral dynamic in swine populations in Chile. Complementary studies are necessary to determine the virulence of this strain and to compare with other lineages present in the country.

Acknowledgments

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Genetic and antigenic diversity of contemporary influenza A virus in swine in Brazil

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Introduction

Respiratory infections caused by influenza A virus (IAV) became frequent in Brazilian swine herds following the introduction of H1N1pdm in 2009. IAV is commonly detected in swine showing mild to severe acute respiratory disease, as the only pathogen, or in association with other viral or bacterial agents (1). Previous investigation revealed substantial genetic diversity of IAVs detected in pigs. H3N2 and H1N2 swine IAV from Brazil were characterized to be most closely related to human seasonal influenza viruses that circulated during the late 1990s and early 2000s. Viral diversity increased after reassortment with co-circulating H1N1pdm09 virus internal genes (2). The objective of the present study was to continue influenza monitoring in pigs to characterize contemporary genetic and antigenic evolution of this important respiratory pathogen.

Materials and Methods

Nasal swabs and lung samples collected from commercial swine in herds located in seven Brazilian states were sent to a veterinary diagnostic laboratory for screening of respiratory agents involved in the porcine respiratory disease complex. IAV positive samples by RT-PCR were submitted for virus isolation in SPF chicken eggs and/or MDCK cells, and genetic sequencing. RNA was extracted from pig samples and the eight gene segments were amplified by RT-PCR using PathAmp FluA reagents. DNA libraries were prepared and submitted for sequencing using Ion Torrent system. Influenza genomes were assembled using Newbler v.2.9.

H1 and H3 hemagglutinin (HA) gene alignments were generated for these sequences alongside a random sample of global human and swine IAVs downloaded from the Influenza Research Database. For each alignment, a maximum likelihood phylogeny was inferred, and statistically supported clades of viruses that demonstrated onward transmission in Brazil were identified. An HA1 amino acid consensus was determined for each clade, and representative strains were identified for antigenic characterization. The representative H1 and H3 IAV were tested by hemagglutination inhibition (HI) using a panel of swine sera against global swine and human seasonal IAV, with newly generated monovalent swine anti-sera against Brazilian swine IAV (3).

Results

From 2009-18, a total of 1010 samples were collected from nursery pigs from farms located in southern, midwestern, and southeastern Brazil. Four hundred and twenty-three (423) samples were positive for IAV by RT-PCR. Ninety-nine samples were isolated and submitted for sequencing, 41 H1N1, 30 H1N2, and 28 H3N2. 38 H1N1 genes were related to the 2009 pandemic (H1N1pdm09) but were derived from at least 9 human-to-swine transmission events from 2009 to present, and included a clade (n=23) of viruses that demonstrated sustained transmission in Brazilian swine. 24 H1N2 and 3 H1N1 viruses were detected and these fell within three distinct human-to-swine transmission events prior to 2009, and were similar to human seasonal H1 viruses circulating between 2000 and 2005. The 28 H3N2 viruses comprised three statistically supported clades, all derived from a single human-to-swine transmission event in the mid-1990s with subsequent genetic evolution. Of the 27 H1N2 strains that we were able to whole-genome sequence, six H1N2 viruses had seven gene segments derived from H1N1pdm, and one segment (NA gene) derived from human-seasonal H3N2 virus. The remaining IAV genomes sequenced so far had H1N1pdm internal genes.

Antigenic maps were generated from the HI data. Antigenic distances (1 AU equals a 2-fold loss in HI titer) demonstrated significant variability among IAV within each clade, and at least 4 AU distance from putative human-seasonal precursor viruses to representative circulating swine strains.

Conclusions and Discussion

These data demonstrated the role and importance of human-to-swine transmission in the evolution and diversity of swine IAV in Brazil. Five co-circulating clades of viruses were identified within three subtypes. Antigenic characterization of representative isolates suggested that Brazilian swine IAV are regionally unique and swine vaccines may have limited efficacy. The swine IAV also demonstrated antigenic divergence from human seasonal strains and therefore may also pose a zoonotic risk.

Acknowledgments

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Homologous protection against Seneca Valley virus in pigs previously exposed to the virus and potential immune correlates

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Introduction

Seneca Valley virus (SVV) causes vesicular disease in pigs, which is clinically indistinguishable from vesicles caused by FMDV and SVDV (1). Field observations have suggested that the SVV-associated disease is self-limiting and previously infected pigs are immune to re-infection (2). It is not well understood what contributes to the protective immunity against SVV and how long it lasts. This study aimed at assessing time course of viremia and antibody development in mature pigs over 100 days after experimental SVV infection and the immune status to the subsequent homologous challenge.

Materials and Methods

Thirteen 9-month-old gilts were inoculated intranasally with a 2015 US SVV strain ($5x10^7$ $TCID_{50}$/pig) (3). On 111 or 124 days post-inoculation (dpi), 11 pigs were re-challenged with the same SVV strain in the same manner as the first inoculation. The pigs were monitored for apparent clinical signs including vesicles throughout the study period.

Sera were periodically collected from all pigs until 33 dpi, on either 111 or 124 dpi right before the re-challenge, and 7 days post-challenge (dpc), as well as at the necropsy between 13 and 43 dpc. The serum samples were tested for viremia by RT-qPCR. Antibody responses, including isotypes, were assessed by serum-virus neutralization (SVN) and indirect fluorescent antibody (IFA) tests. The reactivity of the antibody with four structural proteins (VP1, VP2, VP3, and VP4) of SVV was characterized by Western immunoblot assay (WIA).

Results

All pigs developed vesicular lesions on either coronary band or snout after the initial inoculation (3). After re-challenge, no pigs developed clinical signs.

Viremia was first observed in all pigs by 2-3 dpi and disappeared after 17 dpi. None of these pigs became viremic after the re-challenge.

Neutralizing antibodies were first detected on 4 dpi, peaked on 8 dpi, and were still detectable at high levels in all pigs on the day of re-challenge.

SVV-specific IFA antibodies of all isotypes (IgM, IgA, and IgG) were detected by 7 dpi. IgM started to decline after 10 dpi and was no longer detectable by 33 dpi. A high level of IgG and IgA remained in all pigs on the day of re-challenge (Table 1).

WIA demonstrated that only antibodies against VP2 persisted in all 11 pigs from 7 dpi until the day of re-challenge whereas antibodies against the other structural proteins of the virus were only detectable in some animals on the day of re-challenge. The number of animals seropositive for VP1, VP3 and VP4 continued to decline after re-challenge while all the animals remained seropositive for VP2 until the end of the study (Table 1).

Table 1. Proportion of pigs with detectable viremia, SVN activity, and antibody specific for viral structural proteins at and after the re-challenge

Test		Days post-challenge (dpc)		
		0*	7	13 to 43
RT-qPCR		-§	-	-
SVN		11/11	10/10†	10/10
IFA	IgM	-	-	-
	IgG	11/11	10/10	10/10
	IgA	11/11	10/10	10/10
WIA	VP1	4/11	4/10	1/10
	VP2	11/11	10/10	10/10
	VP3	7/11	6/10	2/10
	VP4	8/11	8/10	6/10

*Eleven pigs were challenged with homologous SVV at dpi 111 or 124, all of which correspond to 0 dpc

§Negative result in all animals tested

†One animal died of excessive bleeding at 2 dpc due to unknown causes.

Conclusions and Discussion

Experimental SVV infection via intranasal route elicited neutralizing antibody response early after exposure. This humoral immune response cleared SVV viremia and established sterile protective immunity in adult pigs against a subsequent homologous challenge.

The protective immunity lasted at least 4 months from the initial infection, if not longer, under the conditions presented in the study.

Serum IgA and/or IgG might be responsible for the anti-SVV neutralizing activity. Furthermore, the VP2 protein of SVV appears to be immunodominant and may contain a major neutralizing epitope(s) playing a role in protective humoral immunity.

Acknowledgments

The study was supported in part by funding from the USDA Cooperative Agreement.

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Virus and antibody dynamics in sows and their offspring after experimental Seneca Valley virus infection during late gestation or perinatal period

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Introduction

Seneca Valley virus (SVV) causes vesicular disease in pigs, which is clinically similar to FMD (1). Increased neonatal mortality has also been reported in breeding farms affected by SVV (2,3). On those farms, piglets during their first week of life presented muscular weakness, lethargy, excessive salivation, cutaneous hyperemia, neurologic manifestations, and diarrhea while some died suddenly (4,5). Since neonatal mortality may be due to in-utero or perinatal infection, this study was conducted to access clinical outcomes and parallel serologic profiles of dams and their offspring after experimental SVV inoculation during late gestation and perinatal period.

Materials and Methods

Ten pregnant sows were inoculated intranasally with a 2015 US SVV strain at different time points either late in gestation (n=5) or within a few days of farrowing (n=5). Piglets born from sows inoculated after farrowing were inoculated orally with the same virus strain at the same time. All animals were monitored for clinical signs. Sera were periodically collected from either at the birth before ingesting colostrum or 0 day post-inoculation (dpi) until 13 to 15 days postpartum (dpp) or dpi. The sera were tested for viremia by RT-qPCR and for antibodies by IFA and SVN assays.

Results

Only one sow which was inoculated on 7 dpp developed snout vesicle while viremia was detected in all of the inoculated sows within 14 dpi. However, there was no significant mortality and gross lesions observed in any of their offspring.

Piglets inoculated with SVV after birth were viremic until 14 dpi and started to develop SVN and IFA antibodies within 7 dpi regardless of the day of the inoculation.

Piglets born to the sows inoculated with SVV during late gestation showed different profiles depending on when the sows were exposed to the virus (Table 1). Piglets from sows inoculated either 18 or 12 days before farrowing were not viremic throughout the study. They were all seronegative at the birth except one animal but they became all seropositive by 14 dpp. Piglets from a sow inoculated 10 days before farrowing were viremic at birth. Most of these piglets also had SVN and IFA antibodies at birth and remained seropositive until the end of the study. Piglets from sows inoculated either 4 or 3 days before farrowing were first viremic on 4 dpp. Antibodies were first detected on 7 dpp in their sera.

Table 1. Proportion of different test positive piglets born from five sows inoculated with SVV in the late gestation period

Days from inoculation to farrowing	Test	Day of bleeding after birth		
		Birth	6-7	13-15
18	PCR	-*	-	-
	SVN	-	6/7	7/7
	IFA	-	7/7	7/7
12	PCR	-	-	-
	SVN	1/14	8/8§	10/10
	IFA	-	8/8	10/10
10	PCR	4/4†	1/5	-
	SVN	3/4	5/5	5/5
	IFA	3/4	5/5	5/5
4	PCR	-	9/9	-
	SVN	-	8/9	9/9
	IFA	-	3/9	9/9
3	PCR	-	8/8	-
	SVN	-	6/8	7/7‡
	IFA	-	-	7/7

*All pigs in the litter were negative.
§Four piglets died with 24 hours after birth and blood samples could not be obtained from 2 piglets at 7 dpp.
†Blood could not be obtained from one piglet at birth.
‡One piglet was euthanized at 9 dpp due to a lack of vigor.

Conclusions and Discussion

No abnormal increase in neonatal mortality was observed in piglets inoculated with SVV directly or born to the sows inoculated with the virus under study conditions. However, this study demonstrated that transplacental SVV infection occurred during late gestation.

Acknowledgments

The study was supported in part by funding through the USDA Cooperative Agreement.

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Eradication of Aujeszky's Disease in a large-scale pig farm in China

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Introduction

Pseudorabies Virus (PRV) significantly reduces profitability in large-scale pig farms. A new variant strain has been found in China since 2011. In 2016, an outbreak of pseudorabies (also known as Aujesky's disease) was reported in a large-scale pig farm in southeast China, caused severe abortions and young piglet mortality. The purpose of this study was to eradicate the PRV from the farm and to evaluate if the various measures applied were effective.

Material and methods

The new PRV variant strain in this farmwas identified based on the results of sequencing. Serum samples from different groups were collected and performed gE-specific ELISA (Svanova) test. Several PRV eradication measures were implemented in this farm: 1) for breeding pigs, two mass vaccinations were conducted with a 3-week interval, followed by regular mass vaccination every 3 months with Ingelvac® Aujeszky MLV; 2) for commercial herds, all pigs received one dose of intranasal immunization at 0-3 days of age; subsequently intramuscular immunization was administrated at 8 and 11 weeks of age, respectively; 3) gE antibody ELISA test (Svanova) was used to monitor PRV infection; 4) farm biosecurity and management procedures were improved by using of an Comprehensive Online Management and Biosecurity Assessment Tool (COMBAT).

Results

Testing results showed that 80% of sows from the breeding herds were gE antibody positive, and seroconversion of growing pigs occurred at 12 weeks of age. Following implementation of a comprehensive eradication program, PRV gE antibody positive rate reduced remarkably. In Jul 2017, no seroconverted pigs could be detected in sows under 7 parities. From Oct 2017 until present, all pigs were gE antibody negative in this farm, which means no more wild strain virus infection afterward. Meanwhile, production performance significantly improved in 2017 compared with the previous year. The farrowing rate increased from 87.4% to 88.2%, and the average farrowing litters/sow/year also rose from 2.19 to 2.26; The survival rate of suckling, nursery and finishing pigs increased 2.86% (to 92.51%), 1.2% (to 97.53%) and 0.7% (to 98.82%), respectively. Collectively, MSY of this farm improved from 17.44 to 18.72.

Conclusion

Significant improvement of pig production performance were found after eradication of PR. According to this successful case, together with management and biosecurity improvement, we found Bartha-K61 strain PRV vaccine (Ingelvac® Aujeszky MLV) can still play an effective role in PRV eradication, despite the existence of variant strains.

Proteasomal degradation of nonstructural protein 12 by RNF114 suppresses porcine reproductive and respiratory syndrome virus replication

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) represents a significant threat to the worldwide swine industry, and the development of effective and sustainable measures to control PRRSV transmission remains a pressing problem (1,2). The function of PRRSV nonstructural protein 12 (Nsp12), which might play essential roles in viral replication and production, remains unknown (3, 4). Investigation of host–virus interactions provides creative insights into potential PRRSV-control methods. RING finger protein 114 (RNF114) belongs to the family of RING-domain E3 ligases (RING E3s), with studies implicating RING E3s in significant roles in host responses to viral infection and replication (5,6). However, its ability to modulate PRRSV infection and the associated mechanisms have not been reported. In the current study, we aimed to explore the relationship between RNF114 and PRRSV replication.

Materials and Methods

Porcine alveolar macrophages (PAMs), MARC-145 cells, and HEK293T cells were cultured. The highly pathogenic PRRSV vHuN4 (GenBank accession no. EF635006) was used in all experiments. MARC-145 cells were transfected with pCAGGS or pCAGGS-RNF114-HA, followed by vHuN4 infection [multiplicity of infection = 0.1]. Cells were harvested at 12-, 24-, 36-, and 48-h post-infection and analyzed by western blot (WB) using an anti-N protein polyclonal antibody (1:1000) produced in our lab. Total RNA was extracted using an RNeasy mini kit (Qiagen), and a PrimeScript first-strand cDNA synthesis kit (Takara) was used for reverse transcription, with SYBR Premix Ex Taq (Takara) used to quantify levels of *ORF7* mRNA. HEK293T cells were co-transfected with the corresponding plasmids using X-tremeGENE DNA transfection reagent (Roche). Co-immunoprecipitation and confocal imaging assays were performed using anti-Nsp12 antibody, rabbit anti-HA monoclonal antibody, and mouse anti-C-Myc monoclonal antibody (Cell Signaling Technology). For Ubiquitination assay, pCAGGS-Nsp12-Myc and pFlag-Ub were co-transfected into HEK293T cells together with or without pCAGGS-RNF114-HA. At 24 hpt, cells were treated with 10 μM of the proteasome inhibitor MG132 or 20 mM of the lysosome inhibitor CQ (Sigma-Aldrich) for 12 h and lysed, followed by respective addition of agarose conjugated with the anti-Myc monoclonal antibody (Sigma-Aldrich). Following overnight incubation at 4°C, the samples were examined by WB.

Results

WB, quantitative real-time polymerase chain reaction, and viral titer assays indicated that RNF114 overexpression significantly suppressed PRRSV replication, whereas *RNF114* knockdown increased viral titer and nucleocapsid protein levels. Additionally, we observed that PPRSV infection led to increased RNF114 levels during the middle and late phases of infection in both PAMs and MARC-145 cells. Moreover, screening of PRRSV Nsps showed that RNF114 interacted with viral Nsp12, and that RNF114-specific anti-PRRSV effects were associated with its ubiquitin ligase activity associated with K27-linked polyubiquitination and degradation of Nsp12 through a proteasome-dependent pathway. These findings identified RNF114 as a critical regulator of PRRSV replication and offer insights into the roles of Nsp12 in PRRSV pathogenesis.

Conclusions and Discussion

In summary, we demonstrated that RNF114 exhibits significantly anti-PRRSV activity via K27-linked polyubiquitination and degradation of viral Nsp12. These results promote further study of members of the RING-UIM E3 family possibly involved in resistance to PRRS transmission and broaden the understanding of host–PRRSV regulatory mechanisms.

Acknowledgments

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Detection of apotential route of PRRSV infection in largescale farrow- to –finish swine farms

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Introduction

The main feature of the large-scale pig breeding farms in Hungary is that 80-85% of them arerun in farrow-to-finish type. In our experience, the most common cause of infection of progeny in these farms is reinfection from older to younger generations.In our study we aimed to answer the following questions. Is it worthwhile to monitor the progeny to detect the presence of wild-type virus at weaning?

Materials and methods

Our studies were carried out on three large scale pig farms of 2000, 1400 and 870 sows, respectively. Farm A is farrowing-to-wean type, while both two rest Farm B and C are farrowing-to-finish ones. Farm A was infected in 2018 by PRRS virus, while in Farm B and C it happened several years earlier. Porcilis® PRRS vaccine (MSD) was used to stabilise herd PRRS status in all three stocks.At the farms, blood samples were taken during the weaning from one piglet/each litter of the selected period. In the second part of the study, we switched to the examination of three piglets/each weaned litters.The blood samples were subjected to PRRS serology (ELISA) andPCR. If indicated the presence of either wild-type or vaccine virus, sequencing was also performed.Based on these data, we have tried to determine whether there is a sow that is shedding wild virus after the stabilization (two-time blanket vaccination) of the herd, and if so, in what proportion?

Results

Three months after mass vaccinations, 1 (0.093%) of the 1077 farrowing is carrying PRRSV that infected the herd were found in Farm A.
The results of Farm B are summarized in Table 1.

Result of PRRS laboratóry tests at Farm B

Table 1.

Period	Blood sampled weaners	Total PCR +	Total Wild virus +	Total vaccine virus +
before culling wild virus pozitiv piglets farrowd sows	8311	97	78	19
after culling wild virus pozitiv piglets farrowd sows	8466	141	0	141

After introduction of the strict culling of sows that hadwild-type virus positive progeny based on the PCR test from the blood oftheir 3-week-old piglets, none of the wild-type virus could be detected, while in 141 cases (1.67%) the Porcilis® PRRS vaccine (MSD) was found, despite the fact that no vaccinations were applied during lactation.

Results of farm C is shown in table 2.

Result of PRRS laboratóry tests at Farm C

Table 2.

Period	Blood sampled weaners	Total PCR +	Total Wild virus +	Total vaccine virus +
Investigation	3349	6	3	3

Conclusions and Discussion

For PRRS, stabilisation of a given herd is defined as the production of PRRSV-negative piglets from PRRSV-positive sows (1).In our study, we found that in a large scale, PRRS infected pig farms, post-vaccination monitoring of 30 piglets per batch is not sufficient to guarantee virus-free status even in the progeny ofa long time-immunized sow population.
In our opinion, this requires a much larger number of tested animals - in our case at least 3 piglets/litter. The reason for this isthat in the case of sows, after mass vaccination, as much as 1% wild-type virus positivity may occur, and such a monitoring test will detect the infecting, wild-type virus in piglets.
The culling of such litters and sows, together with strictinner biosecurity control measures, may result in a safer way of PRRSV-free progeny of large-scalebreeding farms.

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Genetic diversity of Porcine reproductive and respiratory syndrome virus (PRRSV) from 1996 to 2017 in China

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most devastating diseases of the global swine industry(1). The causative agent Porcine reproductive and respiratory syndrome virus (PRRSV) was first isolated in China in 1996, and has evolved quickly during the last two decades(2). Although the outbreak of African swine fever (ASF) in 2018 has concealed the problems caused by other diseases on pig farms, but given the situation of PRRS in other develop countries, it is reasonable to know the real situation of PRRS epidemic in China, to understand the role of the virus evolution, and to predict major problems in the future.

Materials and Methods

In total, 365 PRRSV sequences were gained from GenBank database, of which 353 strains were from isolated in mainland China in from 1996 to 2017.

The complete PRRSV genomic sequences were aligned with the online MAFFT software and ClustalW in the Lasergene software. Genetic recombination was analyzed with the Recombination Detection Program software.

Results

The results showed that high diversity was found among PRRSV isolates, isolates belonged to Original HP-PRRSV, NADC30-like and Intermediate PRRSV subgroups were the major epidemic PRRSV strains circling in the field and will play the major role in PRRS epidemic in the future. Deletions, insertions, and recombinations have occurred frequently in PRRSV genome, deletions mainly occur in NSP2-coding region. Recombination ratio increased since 2015, and the recombinant strains could be divided into three groups: the Inner group, Extensional group, and Propagating group. The evolutionary directions of the isolates in the Extensional and Propagating groups have changed, 10 routes of recombination in the Propagating group were analyzed and sorted into three types. In type 1, the recombination events occurred in different parts of genome, generating multiple recombinant strains. In type 2, the recombination events occur in a similar position, but the length of recombination position varies . In type 3, the recombination site changed completely after propagation .

Conclusions and Discussion

The total PRRSV isolates in China could be divided into eight subgroups, and strains belonging to the Original HP-PRRSV, NADC30-like and Intermediate PRRSV subgroups will play a major role in PRRS epidemics in the future, especially those recombinant strains that have integrated the advantages of their parental strains. Deletion was the main driving force of PRRSV evolution before 2006, and might have also contributed to the virus evolution in a relatively closed environment or low strain diversity area. However, recombination has played a very important role in virus evolution after 2006 and may cause considerable difficulty in PRRS control in the future.

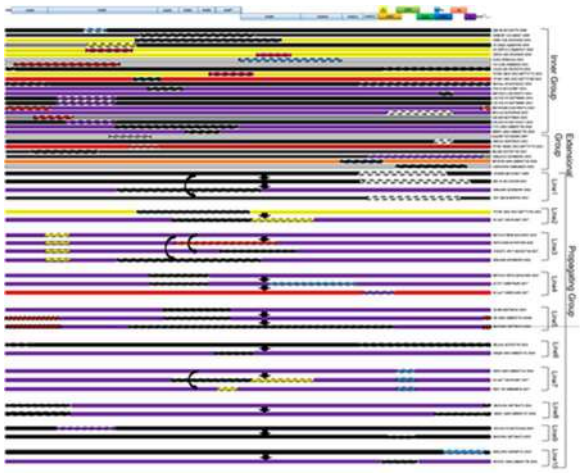

Figure1. Recombinant region in each isolate

EU-type strain, MN184-like strain, NADC30-like strain, Classic NA strain, Intermediate PRRSV strain, Original HP-PRRSV strain, JXA1-P80-like strain, and PRRSV2010 strain are marked with green, orange, purple, blue, gray, black, red, and yellow rectangles, respectively. Major parental strains are shown with solid lines and minor parental strains with dotted lines. The rough sites of recombination are based on strain VR2332.

Acknowledgments

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Comparison of the efficacy of Circovac® with a vaccine applied I.D. under Dutch field conditions

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Introduction

The aim of the study was to evaluate the efficacy of Circovac® against clinical problems related to PCV2 compared to an intradermal (I.D.) PCV2 vaccine (Vaccine A), in the nursery and finisher period under Dutch field conditions.

Materials and Methods

At two conventional herds with 200 and 185 sows, piglets were vaccinated at 3 weeks of age, either with Circovac® (0.5 mL; I.M.) or Vaccine A (0,2 mL; I.D.). Both farms were vaccinating against *M. hyopneumoniae* with Hyogen® (2 ml; I.M.) at the same age. Piglets were randomized per pen and housed in the same environmental conditions. At the age of 12 weeks piglets of both sow herds were moved to the same 2000 finisher farm. The growth performance (ADWG), feed conversion (FCR) and mortality were measured of 4 batches of piglets and 4 other batches of finishers. Per treatment group, the weighted average ADWG, FCR and mortality was calculated.

Results

In total 989 nursery piglets were included in the study. The Circovac® vaccinated piglets showed an ADWG of 479 g/d (n=546) and the Vaccine A group showed 481 g/d (n=443). The FCR was 1,93 and 1,92 respectively and the mortality was 3,16% and 2,20% respectively.

Table 1 Mortality, ADWG and FCR of the nursery pigs (A = vaccine A group; C = Circovac® group).

	No. of pigs	Mortality (%)	ADWG (g/day)	FCR
A	**546**	**2,2**	**481**	**1,92**
C	**443**	**3,16**	**479**	**1,93**

In total 838 finishers were included in the study. The Circovac® vaccinated pigs showed a FCR of 2,33 (n=410) compared to the Vaccine A group
which showed a FCR of 2,41 (n=428). No differences were found for the ADWG which was 858 g/d for both groups and for the mortality which was 0,73% and 0,93% for the Circovac® and Vaccine A group respectively. Only a significant difference in favour of the Circovac® group was found in the FCR ($p<0,05$) in the finishing period.

Table 2 Mortality, ADWG and FCR of the finishing pigs (A = vaccine A group; C = Circovac® group).

	No. of pigs	Mortality (%)	ADWG (g/day)	FCR
A	**428**	**0,93**	**858**	**2,41**
C	**410**	**0,73**	**858**	**2,33**

Discussion and Conclusions

In conclusion, the 2 piglet vaccines demonstrated the very similar efficacy in terms of ADWG and mortality. The FCR was significantly better for the Circovac® group in the finishing period.

Comparison of the efficacy of Circovac® with another commercial vaccine under field conditions

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Introduction

Porcine circovirus type 2 (PCV2) is the primary causative agent of porcine circovirus associated disease (PCVD). Vaccination against PCV2 is standard practice in pig production and a number of different vaccines are available. The aim of the study was to evaluate the efficacy of Circovac® against the effects of a PCV2 infection compared to the commonly used PCV2 vaccine in the Netherlands, in terms of growth performance and mortality under Dutch field conditions.

Materials and Methods

At a conventional health 500 sow herd, with a known PCV2 infection, piglets were vaccinated at 3 weeks of age either with Circovac® (0.5 mL, I.M.) or Vaccine A (1mL, I.M.). Two batches of piglets were included in the study. Piglets were randomized per pen and housed in the same environmental conditions. The 9-10 weeks old piglets were moved to a 4000 places finisher farm. Growth performance (ADWG) and mortality were measured.

Results

In total 572 pigs were included in the study. In the first and second batch the Circovac® vaccinated pigs showed an ADWG of 871 (n=102) and 841 g/d (n=143) and a mortality of 1.90% and 3.90% respectively. The Vaccine A group showed an ADWG of 857 (n=151) and 843 g/d (n=158) and a mortality of 2.58% and 1.27% respectively. There were no statistical differences in the mortality (χ^2 test, $p>0,05$) between the treatment groups.

Table 1: ADWG (g/day) in the finishing unit.

	Circovac®	Vaccine A
Batch 1	871	857
Batch 2	841	843
Weighted Avg.	853	849

Table 2: Mortality (%) in the finishing unit.

	Circovac®	Vaccine A
Batch 1	1.90	2.58
Batch 2	3.90	1.27
Weighted Avg.	3.09	1.92

Discussion and Conclusions

In conclusion, the 2 piglet vaccines demonstrated very similar efficacy in terms of ADWG and mortality in the finishing herd. This study performed under Dutch conditions showed that Circovac® piglet vaccination leads to a solid protection against PCVD and its impact on performance.

Shift to PCV2d predominance in PCVD cases in England and Wales

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Introduction

Confirmed cases of porcine circovirus disease (PCVD) in Great Britain declined to a low level after porcine circovirus 2 (PCV2) vaccines became available. However, PCVD is still occasionally diagnosed. We have previously reported the findings of a genotyping study to characterise PCV2 associated with confirmed PCVD cases in England and Wales from 2011 to January 2016 (n=65) and from which we reported the first detection and emergence of PCV2d (1). Here, we report results of continued surveillance of PCV2 genotype associated with confirmed PCVD cases in England and Wales.

Materials and Methods

PCV2-containing samples derived from 24 cases of PCVD confirmed by the Animal and Plant Health Agency (APHA)'s diagnostic service between April 2016 and September 2019 were analysed. Respiratory disease, wasting and found dead were the most frequently reported clinical signs in the groups of pigs in which the 24 PCVD cases were diagnosed.

DNA was extracted from sections from paraffin-embedded tissue blocks (PETB) as described previously (2) or from fresh tissues using the DNA Mini kit (Qiagen). A fragment of the PCV2 genome (~760bp), encompassing the entire coding sequence of ORF2 was amplified using published primers (3) and characterised by Sanger sequencing: phylogenetic analysis of ORF2 is representative of whole-genome analysis (4).

Results

ORF2 sequence was obtained from 20 of the 24 submissions (Table 1). All but four of the 20 ORF2 sequences were 705 nucleotides in length and encoding a protein of 235 amino acids. Nucleotide sequence identity of these 16 sequences ranged from 97.7 to 100 per cent (10 unique sequences). Phylogenetic analysis showed that these sequences clustered with reference PCV2d strains, and specifically within group PCV2d-2 (5). Four sequences were 702 nucleotides in length, with nucleotide sequence identity ranging from 98.7 to 99.4 per cent. These sequences clustered within genotype PCV2b.

Table 1. Characterisation of ORF2 of PCV2 in cases of PCVD in England and Wales

Year	Number of PCVD cases from which sequence obtained	Number of cases with PCV2b detected	Number of cases with PCV2d-2 detected (unique*)
2016	3	1	2
2017	4	1	3
2018	8	1	7 (5)
2019	5	1	4 (2)
Total	20	4	16 (10)

* Number of unique sequences per year if different to the number of sequences.

Conclusions and Discussion

Our original genotyping study of PCV2 in PCVD cases in 2011 to January 2016 found that the majority of sequences were genotype PCV2b but four sequences were PCV2d-2, detected in 2013/2014 (1).

The current study found the majority of sequences were genotype PCV2d-2. Only PCVD cases diagnosed by APHA were investigated in this study and the data cannot be used as a correlate for the national herd. However, the cumulative dataset from the two studies does appear to indicate progression of a genotypic shift from PCV2b to PCV2d in England and Wales, with PCV2d-2 becoming predominant. This is consistent with reports elsewhere in the global pig population.

The significance of this genotypic shift is uncertain. There are conflicting reports with respect to genotype differences in virulence. PCV2a-based vaccines have been shown to be effective against PCV2d challenge under experimental conditions (6). PCVD cases in this study were mainly diagnosed in either unvaccinated herds or in groups of pigs where an issue was identified with the vaccination regime.

Although vaccination is effective in controlling PCV2-associated disease, it does not prevent virus infection establishing; therefore, PCV2 will continue to circulate in the pigs albeit at a lower rate. APHA will continue to monitor the clinical and pathological details, and PCV2 genotype of PCVD cases diagnosed.

Acknowledgments

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PRRSV antagonizes PDCoV replication and lightens the diarrhea via "bystander" effect

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Introduction

porcine reproductive and respiratory syndrome virus (PRRSV) is well characterized for its immunosuppression in pigs. The coinfection with PRRSV usually leads to more serious symptoms and pathological lesion, caused by the pathogens, such as PCV2, HPS, and *S. suis*. Porcine deltacoronavirus (PDCoV), is an emerging enteropathogenic coronavirus that causes diarrhea in suckling piglets(2, 4). Some PDCoV outbreaks have been found on PRRSV positive farms, however, if the coinfection of PRRSV can exacerbate the diarrhea caused by PDCoV is still unclear. The objective of this study was to analyze the interaction between PRRSV and PDCoV in the pig co-inoculation model.

Materials and Methods

18-day-old SPF piglets, negative for PRRSV and PDCoV, were randomly divided into four groups (PRRSV, PDCoV, PRRSV and PDCoV coinfection, mock control) with 6 piglets in each group, separately raised in 4 rooms. The piglets in PDCoV single- or co- infection groups were orally inoculated with 2 ml 1x10^8 $TCID_{50}$/ml PDCoV (CHN-HN-1601) at 0 day post-inoculation(DPI), and the piglets in PRRSV single- or co- infection groups were intranasally inoculated with 2 ml 1x10^5 $TCID_{50}$/ml NADC30-like PRRSV (CHsx1401) at 3 DPI. 2 ml DMEM was used as mimic inoculum for the rest groups for control. The clinical symptoms were daily recorded. Both nasal and rental swabs, as well as sera were collected at 0, 3, 5, 7, 14 and 21 DPI. 3 piglets from each group were necropsied after euthanasia, at 8 DPI and 21 DPI. The macro- and micro-lesions on lungs and intestines, as well as viral titer and RNA copies were investigated as previous description (3). And the cytokines of TNF-α, IL-1 and IL-6 in the intestinal microenvironment were tested by commercial ELISA kit.

To further explore how PRRSV to antagonize PDCoV replication, the supernatant from PRRSV (CHsx1401) infected PAMs was collected to add into the IPEC-J2 cells culture medium to investigate the inhibition effect on PDCoV replication *in vitro*. The concentration of TNF-α, IL-1 and IL-6 in the PAMs' supernatant and their inhibition effect on PDCoV replication were further tested, through ELISA kit and individually adding into the IPEC-J2 culture medium, respectively. The PDCoV replication was evaluated via titration and testing viral protein expression by western blotting.

Results

4 of 6 piglets in PDCoV single infection group showed severe diarrhea from 4 to 8 DPI, while there was no diarrhea observed in the rest three groups. The scores of clinical symptom, necopsy and histopathologic lesions of intestines, indicated that the piglets in coinfection group showed lighter symptoms and intestinal lesions, compared with those in PDCoV single infection group. Consistently, the PDCoV viral RNA copies in intestine samples of coinfection group were almost 100 times lower. And the TNF-α, IL-1 and IL-6 in both PRRSV single- and co-infection groups were obviously higher (almost 6, 5 and 4 times, respectively; $p<0.01$) than those in PDCoV single infection or mock groups. Meanwhile, no difference in PRRSV caused symptoms and tested results were observed between the two PRRSV infection groups.

In vitro, PDCoV growth was inhibited in IPEC-J2 cells, treated with supernatant from PRRSV infected PAMs, with 100 times of viral titer drop, compared with that from uninfected PAMs. As well the inhibition was carried out during virus replication in cell, but not on the virus attachment/entry and release. And the TNF-α, IL-1 and IL-6, expressed in PRRSV infected PAMs, were further identified to be able to inhibit PDCoV replication via dose dependent way, and the inhibition effect of supernatant could be counteracted by adalimumab, a blocking antibody against TNF-α.

Conclusions and Discussion

In this study, an unexpected antagonizing effect of PRRSV on PDCoV replication both *in vivo* and *in vitro* were initially identified. Considering PRRSV and PDCoV do not share the same target cell, the cytokine might be the "bridge" of their interaction. So, their existence in PRRSV infected supernatants and intestinal microenvironment were tested. And the PDCoV inhibiting function of TNF-α, IL-1 and IL-6, whose antiviral effect on other virus have been reported(1), were further confirmed.

These consistent data above indicate that the cytokines such as TNF-α, IL-1 and IL-6 induced by PRRSV in PAMs could play an important role in intestinal microenvironment to antagonizes PDCoV replication via "bystander" effect, leading to a lightened diarrhea output in PRRSV and PDCoV coinfected animals. However, if this antagonist effect occurs in PDCoV outbreak herd, still need further clinical investigation.

Acknowledgments

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Use of PRRS ELISA to measure antibody response on oral fluids of PRRSV-vaccinated growing pig groups and to associate with mortality

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Introduction

Porcine reproductive respiratory syndrome virus infects swine populations and causes a tremendous economic impact to the global swine industry (1). It was estimated that 55% of the economic impact occurs during the growing phase (1). Thus, there is a need to improve strategies to detect and manage PRRSV infections in growing pigs. Thus this study aimed to demonstrate the use of a PRRS ELISA test to monitor antibody response on oral fluids of vaccinated growing pig groups and associate it with cumulative grow-finish mortality.

Materials and Methods

This retrospective cohort field study monitored the antibody response of 26 growing pig groups, by testing 6 oral fluids at 4 and 7 weeks of age, using the IDEXX PRRS Oral Fluids Ab Test.

The average antibody response of the six samples, expressed as sample-to-positive (S/P) ratios, was classified into "high" (S/P ≥ 5) or "low" (S/P < 5).

Study group 1 (HH) included pigs with high antibody responses at weeks 4 and 7. Study group 2 (HL), pigs with high antibody response at week 4, but low at week 7. Study group 3 (LH), pigs with low response at week 4, but high at week 7. Study group 4, (LL) had pigs with low responses at weeks 4 and 7.

While 13 groups received only one dose of attenuated PRRSV vaccine (21 days old), other 13 groups received two doses (21 and 49 days old).

Growing pig group was the experimental unit. Number of deaths from wean to market, and number of pigs started were collected for each pig group. Mortality rate was the outcome of interest, and thus compared among study groups using Poisson regression. Results were reported by different vaccination protocols.

Results

The study group 4 (low antibody response on both sampling points), was associated with the lowest mortality. Study groups 1 and 2, which were characterized with high antibody response at week 4, had significantly higher mortality than study groups 3 and 4, which were characterized with low antibody response at week 4 (Table 1).

Groups with high antibody response at weeks 4 and 7 had reduced mortality when vaccinated with two doses of PRRSV MLV vaccine (Figure 1).

Table 1. Mortality comparisons among study groups.

Study Group	1 (HH)	2 (HL)	3 (LH)	4 (LL)
Mean mortality	4.9%[a]	5.2%[a]	4.0%[b]	3.7%[b]
Standard error of mean	0.005	0.005	0.004	0.004
Sample size	10	3	8	5

*Superscript letters indicate significant differences at 0.05 confidence level.

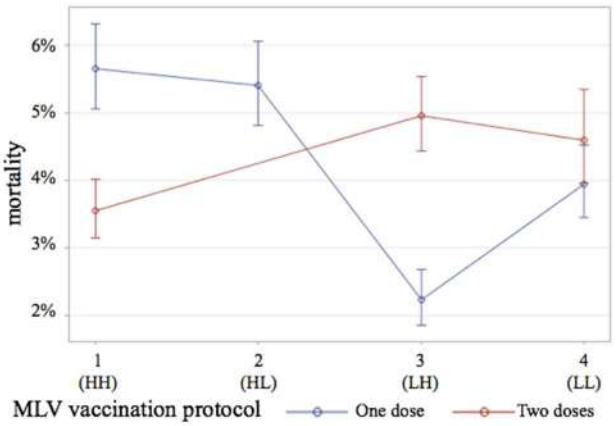

Figure 1. Mortality of study groups by vaccination protocol.

Conclusions and discussion

This study reported an efficient and cost-effective way to monitor antibody response of growing pig groups, using a commercial PRRS ELISA test for oral fluids, and associate it with mortality performance.

High antibody response at week 4 was associated with increased mortality.

Using the two PRRSV MLV doses protocol might help in reducing mortality when groups are characterized with high antibody response at weeks 4 and 7 of age.

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In situ hybridization (ISH) as a diagnostic tool to assess *Porcine circovirus 3* (PCV-3) associated to histological lesions

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Introduction

Porcine circovirus 3 (PCV-3) has been found in sera and tissues from pigs displaying multisystemic inflammation, respiratory and reproductive disease as well as in healthy animals[1,2]. The pathogenicity of this virus has not been clearly established yet, but it has been associated with certain histological lesions in perinatal pigs[3,4]. In the present study, an *in situ* hybridization (ISH) technique was used to consistently detect PCV-3 genome within lesions of pigs showing different pathological conditions.

Material and Methods

Piglets from two cases submitted for diagnosis from a single large indoor farm to the Animal and Plant Health Agency (APHA, UK) were analysed[4]. One comprised four stillborns (two with arthrogryposis), and the second was three 18-day-old piglets with tremors and paresis. All seven displayed multisystemic inflammation[4]. Tissues from these animals tested by PCV-3 qPCR, contained high viral loads (low Ct values).

Formalin-fixed, paraffin-embedded tissues from two piglets in each submission were submitted to IRTA-CReSA to be tested for a newly developed *in situ* hybridization (ISH) technique. ISH was performed as previously described[1] using probes targeting the PCV-3 messenger RNA (mRNA) designed under the RNAscope® technology. Negative and positive tissues were used as controls. A variety of tissues were tested including brain, spinal cord, peripheral nerves, heart, spleen, thymus, lymph nodes, skeletal muscle, kidney, liver and lung. The amount of PCV-3 genome was semiquantitatively assessed following a similar scoring system used for PCV-2[5].

Results

All selected tissues displaying significant histological lesions were positive for PCV-3 genome detection by ISH. The amount of PCV-3 mRNA varied among tissues and structures within tissues, and was scored as moderate to high in all examined animals, in line with the low Ct values of the PCV-3 qPCR in their tissues.

PCV-3 genome was consistently found in arteriolar walls, in a segmental or complete distribution in almost all tissues. Also, PCV-3 genome was found within a significant number of inflammatory cells located perivascularly or within the vascular wall. Nervous tissues had a high amount of labelling mainly in inflammatory cells, but also in meningeal and ependimary cells; neurons were not consistently labelled. Interestingly, white matter in central nervous system (CNS) had higher amount of PCV-3 genome than the grey matter. Macrophage-like cells of different tissues (lungs, liver, lymph nodes) were also ISH moderately positive. Dendritic-like cells in some follicle centers in lymph nodes were also labelled.

Discussion and conclusions

Multiple studies describing PCV-3 detection by PCR have been published in the last 4 years. Some of them have claimed the putative association of this virus with different pathological conditions, but detection of an endemic agent alone is not sufficient for the diagnosis of a disease[3]. In the present report, the use of ISH allowed the detection of moderate to high amounts of PCV-3 genome in association with multisystemic inflammatory lesions in stillborn and sucking pigs (also showing CNS clinical signs)[4]. These results reinforce the potential association of this novel circovirus as a cause of these lesions, as has been recently suggested[3].

In conclusion, the combination of histopathology together with a technique able to detect PCV-3 within lesions such as ISH further supports the putative causal association of this virus with cases of reproductive failure (stillbirth) and multisystemic inflammation in swine.

Acknowledgments

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Porcine circovirus 2 (PCV-2) genotypes found in vaccinated pigs diagnosed with PCV-2-systemic disease

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Introduction

Vaccination of piglets and/or gilts/sows against *Porcine circovirus 2* (PCV-2) is a common practice all over the world (1). Vaccines are able to prevent clinical disease but not infection. However, PCV-2-systemic disease (PCV-2-SD) is occasionally diagnosed in vaccinated farms (2), probably due to early infections which are not able to be prevented by weaning vaccination strategies.

Since a number of PCV-2 genotypes have been circulating in the last decade, the present work aimed to assess which viral genotypes were circulating in these farms experiencing disease in spite of vaccination. The study was performed in Spain during the period 2009-2018.

Material and Methods

A total of 35 cases diagnosed as PCV-2-SD at the *Servei de Diagnòstic de Patologia Veterinària* (*Universitat Autònoma de Barcelona*, Spain) were selected. They corresponded to pigs from PCV-2 vaccinated farms collected between 2009 and 2018, with confirmed PCV-2-SD diagnosed cases (3). These animals displayed clinical signs compatible with the disease (mainly wasting and respiratory distress), moderate to severe lymphocyte depletion and granulomatous inflammation of lymphoid tissues, and moderate to high amount of PCV-2 antigen detected by means of immunohistochemistry. The age of the animals varied between 8 to 16 weeks.

DNA was extracted from the lymphoid tissues included in paraffin blocks, and the whole PCV-2 Cap gene (ORF2) was amplified by PCR (4). PCR products positive for PCV-2 were purified using a commercial kit and sequenced with the ABI PRISM sequencer 3130xl.

The edition of the sequences was carried out using ChromasPro version 2.1.8. The sequences obtained were aligned with representative sequences of different PCV-2 genotypes recovered from the GenBank database using the Clustal Omega multiple alignment method. The phylogenetic tree was constructed according to the Maximum Likelihood method and the Hasegawa-Kishino-Yano model (5).

Results

PCV-2 Cap gene from all cases was successfully sequenced and genotyped. Two different genotypes were retrieved from these cases: PCV-2b (n=18) and PCV-2d (n=17).

Interestingly, only PCV-2b sequences were obtained between years 2009 to 2012 (14/18), while the remaining 4 sequences were found in the period 2015-2017. In contrast, all PCV-2d sequences were found between 2014 and 2018.

Discussion and conclusions

The present study evidenced the dominance of PCV-2b and PCV-2d genotypes associated with cases of PCV-SD in vaccinated farms during the period 2009-2018 in Spain. Although it has been suggested that PCV-2d could be more frequently involved in cases of PCV-2-SD in vaccinated farms, current results do not support such hypothesis.

The higher prevalence of PCV-2b associated disease cases before 2014 and the dominance of PCV-2d afterwards probably reflect the general prevalence of these genotypes over time. In fact, several studies pointed out to a global genotype shift from PCV-2b to PCV-2d at epidemiological level (7). Therefore, it is very likely that obtained results simply reflect the overall epidemiology of this virus and not any association with increased virulence of genotype PCV-2d.

Acknowledgments

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Surveillance for Classical Swine Fever in wild boar in the State of São Paulo

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Introduction

Wild boars are widely distributed in the state of São Paulo, causing serious economic, environmental and social damage (4), in addition to representing an important health risk due to the potential for spreading diseases (1). The Coordination of Agricultural Defense (CDA), the body responsible for animal health in the State of São Paulo, has been dedicated to the serological surveillance of wild boars as an important source of information on the viral circulation of classical swine fever (CSF) in swine populations. This activity was recognized for the important epidemiological role of wild boar in maintaining CSF (1,3, 5) as a reservoir for the virus and a possible source of infection for domestic pigs (1,2).

Materials and Methods

CDA works to build a network of collaborators to collect samples in the field, for this work four training sessions were held, two in São José dos Campos (2015 and 2019), Angatuba (2015) and Jaboticabal (2019) with participation 19 CDA veterinarians and 61 hunters.

The events were promoted by CDA, Ministry of Agriculture, Livestock and Supply (MAPA/SFA/SP) and with the technical collaboration of the specialist in the theme researcher Dra. Virginia Santiago Silva from EMBRAPA Swine and Poultry of Concordia, Santa Catarina .

From then on, surveillance activities began with the collection of blood serum samples carried out at the time of slaughter by the hunters, after which the samples were delivered to the CDA's regional public service units, where they were properly preserved until they were sent to the Swine Diseases "Washington Sugay", from the Biological Institute (IB/SP), and submitted to the Immunoenzymatic Assay through indirect ELISA (CSFV Ab, da INDEXX), for the detection of antibodies against CSF. The period of performance of this work comprises the serological surveillance activities of wild boar between 05/31/2015 to 02/28/2020.

Results

63 blood serum samples were received and 63 laboratory tests were carried out at the IB/SP, all of which were negative for PSC. Table 1 shows the results over the period, with emphasis on the year 2019 when partnerships with universities and research laboratories were carried out significantly increased the samples received.

Table 1. Results ASF of feral boars collected and analyzed samples.

	Samples for years					
	2015	2016	2017	2018	2019	2020
Samples	1	8	3	1	45	5

[1]Samples were collected from hunters in São Paulo State in 2015 at 2020 (partial).

Conclusions and Discussion

We can conclude that these results provide a complementary security that the CSF virus is not circulating in the regions and in the sampled populations, these results added to the other activities of the State Program for Suidae Health (PESS/CDA) strengthen the maintenance of sanitary recognition as a free zone of CSF. In order to achieve a truly robust surveillance system capable of identifying the entry of CSF in wild boars it is necessary that the actions be continuous, lasting and strategically expanded to other regions of the State, through the realization of new partnerships and training, aiming to expand the network of collaborators and thus increasing the representativeness and reliability of the health situation.

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Porcine circovirus type 2 (PCV2) genotypification in Mexico

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Introduction

Porcine Circovirus type 2 belongs to the family *Circoviridae*, genus *Circovirus*, its genome is DNA with an approximate length of 1.9 kb and is formed by two open reading frames (ORF). ORF 1, also called Rep, is the most conserved gene among the PCV-2 genotypes and codes for viral polymerase; ORF 2 or Cap, codes for structural proteins and it is the most variable gene, attributing immunogenic traits and is used for phylogenetic studies. Eight genogroups have been identified circulating around the globe (a-h)[1,2]. In Mexico PCV-2 circulation was identified in 2004 and genogroups a, b and e[3,4] have been described since. The aim of this study was to genotype current PCV-2 circulating in pigs from Mexico.

Materials and Methods

From 138 samples of blood-serum, lung and lymph nodes corresponding to pig farms in the states of Guanajuato, Jalisco, Michoacán, Querétaro and Morelos, collected during 2013-2014 and 2018 - 2019, the extraction of viral DNA was performed and by means of an end-point PCR, the ORF 2 of PCV-2 was amplified. The products obtained were purified and sequenced. Sequences were analyzed using MEGA 7 software; multiple alignments were performed with sequences previously reported in the GenBank obtaining phylogenetic inferences and genotyping analysis.

Results

A total of 36 ORF 2 sequences were obtained, of which 3 of them were grouped in the PCV-2b genogroup and the remaining 33 resulted PCV-2d genogroup as shown in Table 1 and Figure 1. PCV-2b sequences are from Guanajuato and Jalisco, while in all the sampled states, PCV-2d was identified.

Table 1. ORF 2 sequences from sampled states.

State (sample collection year)	PCV-2b	PCV-2d	TOTAL
Guanajuato (2013, 2014)	2	11	13
Jalisco (2014, 2019)	1	9	10
Puebla (2018)	0	7	7
Querétaro (2014)	0	2	2
Michoacán (2014)	0	2	2
Morelos (2019)	0	2	2
TOTAL	**3**	**33**	**36**

Conclusions and Discussion

In Mexico, Bedolla *et al*. and Harmon *et al*., identified genogroups a, b and e from samples between 2013 and 2015. PCV-2b identified in this study corresponds to samples obtained in 2012. PCV-2d, which had not been previously reported in Mexico, was the most frequently identified in samples from 2013 to 2019 (91.67%). Results suggest, that PCV-2d genogroup is the one that circulates most frequently in pig production systems from Guanajuato, Michoacán, Jalisco, Puebla, Morelos and Querétaro. These findings demonstrate the genetic variability of PCV-2 over time as well as being a possible explanation that in some farms with good management of PCV-2 control, which includes an adequate vaccination program, are presenting cases of PCV-2-associated disease confirmed by laboratory tests.

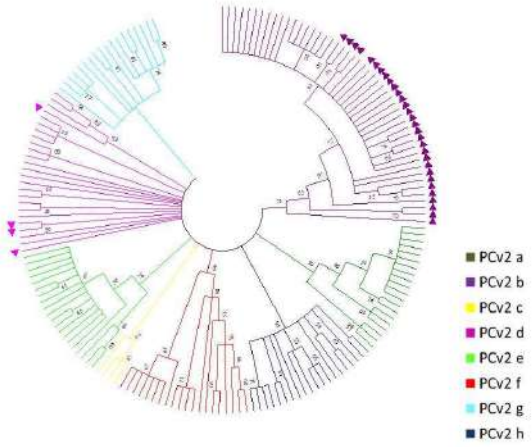

Figure 1. PCV-2 genogroups phylogenetic analysis.

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Characterization of the complete genome of porcine Circovirus type 2 in Mexico

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Introduction

Porcine Circovirus is a DNA virus, which is divided into three large genotypes, Porcine Circovirus type 1 (PCV-1) which has been identified in culture cell lines and is not pathogenic in pigs. Porcine Circovirus type 2 (PCV-2) has been described in association with various diseases and as one of the main causes of economic losses in pig production systems[1]. In 2013, Porcine Circovirus type 3 (PCV-3) was recognized in Asia, nowadays it has spread to Europe and America[2], in Mexico it was reported in 2018[3]. The aim of this study was to molecularly characterize the complete genome of circulating PCV-2 in Mexico through a retrospective analysis.

Materials and Methods

Blood-serum, lung and lymph node samples from 867 Mexican pig farms were evaluated: Guanajuato 144, Jalisco 687, Michoacán 20, Querétaro 10, Puebla 4 and Morelos 1. As a diagnostic test, an end-point PCR was performed into all samples to amplify the 824 bp fragment of PCV-2 Rep gene. Once the positive samples were identified, the entire genome was amplified, and the products were purified and sequenced on the Ion Torrent PGM. The sequences obtained were analyzed in MEGA 7 software.

Results

PCV-2 circulation was confirmed in 138 out of the 867 farms (16%), and positive farms located in the states of Guanajuato, Jalisco, Michoacán, Querétaro, Puebla and Morelos. The frequency detected in these states was 2% to 70%, being the highest during 2012 and 2014 in Michoacán and Guanajuato. Results are shown in Table 1.

Table 1. PCV-2 positive cases of farms in Mexico.

Year	State	Positive cases/total (%)
2012	Guanajuato	50 /72 (69%)
	Michoacán	8/10(80%)
2013	Jalisco	18/ 200(9%)
	Guanajuato	9/ 72(13%)
2014	Jalisco	18/ 221 (8%)
	Michoacán	5 /10 (50%)
	Querétaro	7 /10 (70%)
2015	Jalisco	16 /267 (6%)
2019	Puebla	3 /4(75%)
	Morelos	1 /1(100%)
Total		**138 / 867 (16%)**

The assembly of 21 sequences of the complete PCV-2 genome was performed, four of these sequences presented high homology with sequences reported in Latin America and 17 remaining sequences presented homology with sequences reported in Asia.

Conclusions and Discussion

In 2018[4] Bedolla *et al*., described that the strains identified in different states of Mexico presented a high homology with sequences reported in Asia between 2004 and 2012, our results show that the sequences obtained between 2012 and 2013 have a greater homology with Latin American strains, and those classified as Asian belong to another PCV-2 genogroup not yet described in Mexico. These results suggest a circulation of two different PCV-2 genogroups in pig farms in Mexico, showing that PCV-2 has genetic changes. Future investigation is needed to determine the association of these genogroups with disease presentation in the field.

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PRRSV1 genetic diversity present in an endemically infected farm is driven by the founder variants transmitted by the sows

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) has a continuously expanding genetic diversity. Mutation and recombination are the factors that create viral diversity while selection pressure is a driving force that probably shapes the predominating virus variants. In the herd, transmission may occur vertically or horizontally. In this later case, recipient animals may have some pre-existing immunity or not. Within this framework it would be reasonable to think that constraints to viral diversity will be different in those different cases. The aim of this study is to determine the generation of genetic diversity of PRRSV1 in piglets and weaners of an endemically infected farm and to relate the genetic diversity with the potential transmission chains between the animals.

Materials and Methods

A longitudinal study was performed on a positive unstable farm, where a blanket vaccination protocol with a PRRSV1 MLV was being applied. For the purpose of the study, animals in 10 litters were followed (6 piglets per litter). Umbilical cords were taken immediately after birth and then blood samples were collected at 2, 4, 6, and 9 weeks of age. All included animals were ear tagged and all animal movements between pens were recorded.

The presence of PRRSV1 was assessed by RT-qPCR. A selection of positive samples (n=24) was used for viral isolation in porcine alveolar macrophages and single passaged isolates were characterized by NGS in an Illumina Miseq Platform, from which the viral quasi-species and the complete consensus PRRSV1 genome was obtained.

Additionally, ORF5, nsp2, and nsp9 Sanger sequences were obtained from all the positive PRRSV1 samples (any age) with C_t values lower than 31 (n=86).

The evolutionary relationships among sequences were reconstructed with two phylogenetic analyses: one applying a Maximum Likelihood method with the GTR model and 1,000 bootstrap replicates using the package MEGA7, and a second with a Bayesian inference based on three replicates of 1,000,000 generations each, considering the same evolutionary model calculated with Mr Bayes. The nucleotide diversity (π) was calculated for every segment and sampling time using DnaSP.

Results and discussion

The phylogenetic trees based on complete genome sequences consistently identified clusters of viral variants that could be linked to given vertical transmission events. The chain of infection could be reconstructed from these phylogenetic trees indicating that intra-herd diversity was mainly generated by the vertical transmission. In other words, the founder variant transmitted by a sow could be traced back in the horizontal transmission events until the end of the nursery phase, regardless of whether the transmission happened between siblings, in the nurseries or with or without maternal antibodies. Within the PRRSV1 genome, nsp2, nsp9, and ORF5 were the segments that better identified these phylogenetic groups.

The whole genome p-distances ranged between 0.2-0.7% within the identified clusters, with values around 1% being the highest reported among them. Within PRRSV1 genome, the segments showing the highest p-distances were ORF4 (2.5%) and ORF5a (3.0%). The overall mean distance between the ORF5 sequences was 0,8%, and the highest p-distance reported was 2.1%. Although the number of infected animals was higher at later ages, the nucleotide diversity was not significantly different at different ages in any of the segments considered.

For the nsp2, nsp9, and ORF5, several nucleotide positions were identified as variable in the alignments and allowed us to define conserved marker positions among the phylogenetic groups.

Conclusions

The founder variant transmitted by the sow is key in the generation of PRRSV1 diversity within the farm. Also, the results indicate that the diversity within clusters varies with time, despite the global diversity in the farm does not, since this diversity is driven by the clusters initially transmitted by the sows. Furthermore, the transmission chain can be traced using nsp2, nsp9, and ORF5 clustering.

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Raman microspectroscopy as an alternative diagnostic for porcine circovirus type 2

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Introduction

Porcine circovirus type 2 (PCV2) is a small non-enveloped virus with single-stranded circular DNA and has been associated with different disease syndromes collectively named PCV diseases (PCVD). The detection of PCV2 specific DNA and/or protein in histological lesions is considered a hallmark of PCV2 diagnosis (3). Although these methods are sensitive and specific, they are costly and time-consuming. Raman microspectroscopy (RMS) has been used in virology for rapid and specific identification of viruses such as Influenza, Adenovirus, Hepatitis B and Dengue (1, 2, 4, 5). RMS is a non-destructive fingerprinting technique, which requires no sample preparation, and it is based on the interaction of laser light with the vibrational modes of molecules, thus causing detectable inelastic scattering. This study aimed to demonstrate the primary classification of porcine cells infected with PCV2 and submitted to RMS, presenting promising outputs for future diagnostic applications.

Materials and Methods

Swine testis cells (ST) in 6-well plates were prepared and inoculated in triplicate with PCV2 virus strain (BRMSA 01351). After adsorption, plates were incubated for five days (37ºC, 5% CO_2). Uninfected ST cells were kept as a negative control. D-glucosamine treatment was conducted on day 2 and, on day 3, ST cells were submitted to a synchronization protocol. On day 5, ST cells were fixed with paraformaldehyde (4%) and analyzed in a Raman confocal microscope (InViaTM Renishaw®) equipped with a 633 nm laser. A total of 150 spectra centered at 1300 cm^{-1} was obtained for each sample (5 spectra/cell) of PCV2 infected and uninfected cells, with the acquisition time of 5 seconds. Data were pre-processed for baseline correction, noise reduction, and spectral intensity normalization. A multivariate analysis was performed through Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA), followed by a leave-one-out cross-validation (LOOCV) test.

Results

The averaged spectra obtained from biological replicates of PCV2-infected and uninfected cells analyzed are presented in Figure 1. The spectra are characterized by peaks due to protein, lipid and DNA vibrations. Figure 2 demonstrates the statistical clusterization obtained with the PCA/LDA analysis. The LOOCV test showed 100% of sensitivity.

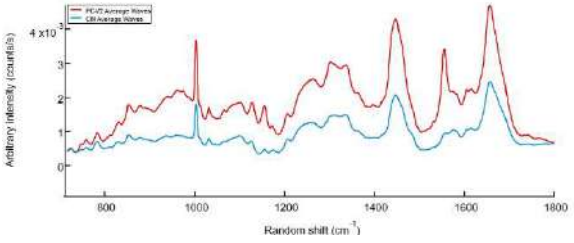

Figure 1. Average spectra from PCV2 infected (red) and uninfected cells (blue).

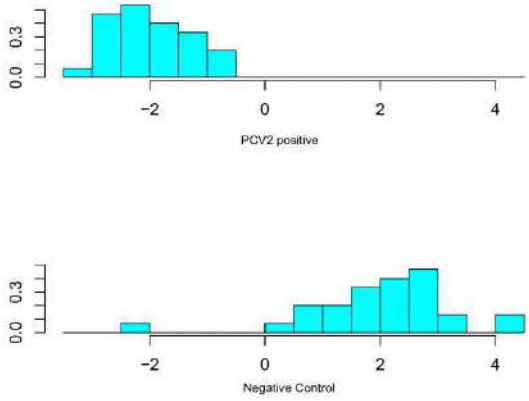

Figure 2. Histogram of sample clusterization obtained with the PCA/LDA applied to RMS data.

Conclusions and Discussion

Based on the high sensitivity observed in our results, we conclude that RMS combined with PCA-LDA analysis is an effective strategy to discriminate PCV2 infected from uninfected cells. It is a promising foundation for diagnostic methods development on preventive veterinary medicine.

Acknowledgments

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Assessment of the nasal virome from respiratory outbreaks with or without the participation of swine influenza virus and randomly sampled herds in Spain

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Introduction

The purpose of the present work was to assess the presence of *Influenza B virus* (IBV), *Influenza D virus* (IDV), *Porcine Parainfluenza virus type 1* (PPV1), *Porcine Reproductive and Respiratory Syndrome Virus* (PRRSV), *Porcine Respiratory Coronavirus* (PRCoV), *Swine Orthopnemovirus* (SOV), *Porcine Circovirus types 2, 3 and 4* (PCV2-4), and *Porcine Cytomegalovirus* (PCMV) in outbreaks of respiratory disease and randomly sampled herds.

Materials and Methods

In the present study, nasal swabs (n=743) were collected from pigs showing fever and respiratory signs as sneezing and coughing from fifty-three farms suffering a respiratory disease outbreak. Samples were initially analyzed by RT-PCR for *Influenza A virus* (IAV). Positive IAV cases (n=23; 404 nasal swabs) and IAV-negative cases (n=30; 340 nasal saws) were compared for the presence of the abovementioned respiratory viruses. Additionally, nasal swabs were randomly collected from 185 weaners of 10 farms (profiles). Samples were pooled in groups of two or three samples and DNA/RNA was extracted by using an automated magnetic particle processor with the MagMAX™ CORE Nucleic Acid Purification Kit (Thermo Fisher Scientific). Presence of IBV, IDV, PPV1, SOV, PCV2-4 and PCMV were evaluated from the RNA by means of real time RT-PCR methods previously described.

Results

IBV, IDV, PPV1 and PCV4 were not detected in the present study. Table 1 summarizes the results obtained in the present study for the pathogens that tested positive. Overall, PCV2 showed the lowest prevalence among the viruses present in the samples ($p<0.05$). PCMV and PCV3 showed higher prevalences compared to other viruses ($p<0.05$). PRCoV was detected in a higher proportion in Pos-IAV outbreaks than in Neg-IAV outbreaks ($p<0.05$). Finally, prevalence of SOV was similar in IAV-positive and negative cases.

Table 1. Number of RT-PCR positive cases and pools from the Pos-IAV, Neg-IAV cases and profiles evaluated in the present work. IBV, IDV, PPV1 and PCV-4 (negative) are not shown.

Virus	Pos-IAV		Neg-IAV		Profiles	
	Cases	Pools	Cases	Pools	Cases	Pools
PRRSV	7/10	27/76	9/12	24/66	5/10	12/65
PRCoV	10/13	47/81	6/18	17/67	NA	NA
SOV	11/23	33/157	6/30	10/133	7/10	27/65
PCV2	10/23	26/157	3/30	9/133	0/10	0/65
PCV3	21/23	99/157	18/30	65/133	7/10	38/65
PCMV	23/23	117/157	15/30	71/133	10/10	50/65

Conclusions and Discussion

Nasal virome of pigs has previously shown to be very complex (1). As seen in the present work, in both respiratory outbreaks and apparently healthy pig populations, viruses that can be usually associated with respiratory problems can be detected. Up to date, the information on SOV prevalence in swine farms is scarce. This virus was firstly detected in both feral and domestic swine in 2016 (1). This is the first detection of SOV in Spanish pig herds. The results observed in the present study indicate a high prevalence of SOV in weaners, particularly in the randomly sampled herds, suggesting that the presence of this virus cannot be related, apparently, with the respiratory disease. Further analysis is needed to evaluate the epidemiology of this virus in pig herds.

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Detection of porcine circovirus type 2 and type 3 by oral fluid samples in swine herds from Brazil

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Introduction

Porcine circovirus 2 (PCV2) is known to cause the porcine circovirus associated diseases (PCVAD), which includes postweaning multisystemic wasting syndrome (PMWS), proliferative and necrotizing pneumonia, granulomatous enteritis, and occasionally porcine dermatitis and nephropathy syndrome (PDNS) and reproductive failure. Worldwide, the main pathogenic subtypes for pigs are: PCV2a, PCV2b, PCV2d, associated with major economic losses in the swine industry. Porcine circovirus 3 (PCV3) is a recently described virus, and has been detected in pigs with different clinical and pathological conditions, mainly reproductive and respiratory disorders, and occasionally multisystemic inflammation and myocarditis. The diagnosis of both viruses infection is made through serum, organs and secretions. Currently, oral fluid has been widely used as it is a less stressful, easy and quick way to collect samples for diagnosis. Initially, the most prevalent PCV2 subtype in Brazil was PCV2a and soon PCV2b became a matter of concern, however, in recent years, PCV2d has been detected in several Brazilian herds (2). Moreover, PCV3 has been shown to be present, alone and in co-infections with PCV2d, in several symptomatic animals in different farms. The present work aims to detect and genotype PCV2 and PCV3 using the PCR technique in routine samples, in order to trace the current profile of the infection in Brazilian swine commercial herds.

Materials and Methods

Samples of oral fluid and organ fragments were collected from eight commercial pig farms in the states of Minas Gerais, Goiás, Mato Grosso, Rio Grande do Sul and São Paulo and sent for diagnosis and genototyping at the Research Laboratory in Animal Virology, UFMG. The samples were collected from May to December 2019, from PCV2 vaccinated and unvaccinated. The samples were obtained from farms that presented clinical disease compatible with PCV2 and PCV3 infection. Forty-three oral fluid samples were collected using the cotton string collection method (3). The samples were immediately refrigerated after collection and frozen until delivery to the laboratory. These samples were the focus of the research for diagnosis, due to the practicality involved in the sampling process. Fifteen samples of organ fragments (lung, lymph node, spleen) from animals showing signs of PCVAD were sent, along with oral fluids. PCR assays were performed using specific primers for each genotype (PCV2a, PCV2b, PCV2d and PCV3) (4,5).

Results

Table 1. Results of PCV2 (a, b, d) and PCV3 genotyping, obtained from samples of oral fluid and organ fragments.

	RESULTS - PCR GENOTYPING					
SOURCE	PCV2 a	PCV2 b	PCV2 d	PCV3	Negative	Co-infection
ORAL FLUID	0/43 (0%)	0/43 (0%)	14/43 (32.56%)	24/43 (55.81%)	13/43 (30.23%)	8/43 (18.6%)
ORGAN FRAGMENTS	0/15 (0%)	0/15 (0%)	8/15 (53.33%)	8/15 (53.33%)	3/15 (20%)	4/15 (26.66%)
TOTAL	0/58 (0%)	0/58 (0%)	22/58 (37.93%)	32/58 (55.17%)	16/58 (27.58%)	12/58 (20.69%)

In all eight sampled farms, viral DNA was detected through PCR, confirming the circovirus circulation. In all farms there were animals that presented clinical disease, where four farms were vaccinated against PCV2 and four farms were not vaccinated. The species and genotypes most present in the farms were PCV2d and PCV3. The PCV2a and PCV2b genotypes were not detected in the samples, suggesting the non-circulation of these viruses in the sampled period. (Table 1)

Discussion and conclusions

The detection and genotyping of PCV2 and PCV3 in oral fluid samples demonstrated the efficiency of oral fluid as a collection material for molecular diagnosis in Brazil, even under inappropriate shipping conditions, with periods of material transport greater than 2-3 days. This efficiency in the PCR test was ensured through the detection of organ fragments, considered as good sample models. PCV2d and PCV3 are circulating in Brazilian pig farms, causing clinical disease, even in vaccinated farms. Oral fluid samples are effective in the detection and genotyping of PCV in Brazil.

Acknowledgments: CNPq and FAPEMIG

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Using the SEQUIVITY™ Dashboard for IAV-S monitoring and vaccination design

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Introduction

Influenza A virus in swine (IAV-S) is a very common and frustrating pathogen that can result in costly losses for swine producers. IAV-S prevention can be difficult given ease of transmission, the human-swine infection potential and high mutation rate. Vaccination against IAV-S is especially challenging due to the vast diversity of the hemagglutinin (HA) proteins present among strains circulating in the swine population. IAV-S undergoes both antigenic shift and drift leading to this extensive diversity.[1] Vaccination success is heavily dependent on sequence analysis that enables strain matching of the HA protein to the field strains that are causing disease.[2,3] MSD Animal Health (MSD) uses the SEQUIVITY™ Dashboard to assist customers with sequence management and vaccine design.[4] Pairing the Dashboard with the SEQUIVITY™ alphavirus replicon technology to produce RNA particle (RP) vaccines enables veterinarians to better protect herds against IAV-S.

Material and Methods

MSD actively monitors IAV-S diversity and how it changes overtime using the SEQUIVITY™ Dashboard. The HA gene sequences of IAV-S for H1 and H3 strains were analyzed using both customer and Genbank data in the U.S. over the past two years. The analysis compared two time periods; December 2017 through December 2018 (1472 H1 and 649 H3 sequences) and December 2018 through December 2019 (1246 H1 and 496 H3 sequences).

Results

The multi-year comparison of IAV-S sequence analysis in the United States is presented in Table 1.

Table 1. IAV-S strain trends over time

Strain	Dec. '17-Dec. '18	Dec. '18-Dec. '19	Change
H3 cluster 4	10.8%	11.4%	0.6%
H3 human-like	19.6%	16.5%	-3.1%
H1 alpha	6.1%	11.8%	5.7%
H1 beta	0.8%	0.3%	-0.5%
H1 pandemic	5.2%	10.3%	5.1%
H1 gamma	30.6%	22.5%	-8.1%
H1 delta 1a	14.6%	5.7%	-8.9%
H1 delta 1b	3.7%	1.3%	-2.4%
H1 delta 2	8.6%	20.2%	11.6%

Important changes identified include a decline of over 8% in both H1 gamma and H1 delta 1a strains. Strains with increased prevalence were H1 alpha and H1 pandemic strains with over 5% each. The greatest increase was found with H1 delta 2 rising from 8.6% to 20.2%. In our experience over the past several years, these findings indicate significant changes in IAV-S circulating field strains that in turn require corresponding alterations in vaccine design.

Discussion

Understanding trends in IAV-S circulation allows customers to make the most informed vaccine strain selection. The most common IAV-S control strategy employed by swine veterinarians is the "reactive/treatment vaccination approach" which occurs when a new IAV-S strain enters a farm causing an increase in clinical disease. Here, the attending veterinarian designs a custom vaccine based on the virus(es) circulating within the farm. Vaccination is then typically applied only after the infection is endemic. This is a costly strategy with many weeks of lost production waiting for the vaccine to be produced and administered to the herd. Using the SEQUIVITY™ Dashboard, veterinarians can move their vaccination strategy to a more "proactive/prevention approach" utilizing the dashboard's geospatial analysis function to include at-risk strains from surrounding herds into the vaccine. This enables veterinarians to move away from a reactive strategy and towards a proactive vaccination program.

Conclusion

The SEQUIVITY™ Dashboard is used to assess the IAV-S diversity in the field. The Dashboard enables the tracking of new and emerging strains, identifying area-dominant strains, and determining trends in the prevalence of existing strains. This monitoring and analytics tool enables MSD customers to make informed strain selections in vaccine design and allows customers to move from a reactive to a proactive vaccination approach.

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Evaluation of PCV2 viremia in pigs vaccinated with Porcilis PCV M Hyo in Taiwanese farms with natural PCV2 challenge

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Introduction

Porcine respiratory disease complex (PRDC) is a major swine respiratory disease found in swine industry worldwide. Porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* (Mhp) are considered to be the most important primary agents that cause PRDC (1). Up to present, vaccination remains the most cost-effective approach to control and prevention of PRDC (1). In Taiwan, more positive rate of the PCV2 antigen found in growing pigs in recent years (5). However, there is limited information of PCV2-viremia in the pigs vaccinated with different PCV2 vaccines under natural PCV2 infected herds in Taiwan. Therefore, the objective of the present study was to evaluate the PCV2-viremia during nursery to fattening stage in the field conditions compared with other commercial vaccine.

Materials and Methods

The study was done in four farrow-to-finish farms in Taiwan. Piglets at 3 weeks old from each herds were randomly assigned to two groups: group A were vaccinated with a commercial vaccine, group B received Porcilis® PCV M Hyo (MSD Animal Health). Blood samples were randomly collected from tezn pigs of each group in each farm at 3, 8, 12, 16, 20 and 24 weeks old. The presence and amount of PCV2 viral DNA in serum was determined by a real-time PCR assay as described previously (5). Real-time PCR results were interpreted as log10 copies per microliter serum for the statistical analysis. The amount of PCV2 load higher than 10^4 copies number per microliter was used to categorize pigs as porcine circovirus-associated diseases (PCVAD) (2, 3, 4). The area under the curve (AUC) of PCV2-viremia for each group in each farm was calculated using the log transformed values of the individual pig PCV2 load over the time of this study.

Results

The overall PCV2-viremia over time for both groups are shown in Figure 1. At 16, 20 and 24 weeks of age, pigs from group B had a significantly lower PCV2 load than that from group A. When AUC was analyzed for each group in the different farms, It is observed that no significant differences (P>0.05) were found between these two groups in both Farm 2 and 4. In contrast, AUC of the group A was highly significant higher than group B in both Farm 1 and 3 (P<0.0001) (Table 1).

Conclusions and Discussion

Results of PCV2-viremia were significantly lower in growing pigs vaccinated with Porcilis® PCV M Hyo at three weeks of age. The overall PCV2-viremia over time was the lowest in pigs from Porcilis® PCV M Hyo group.

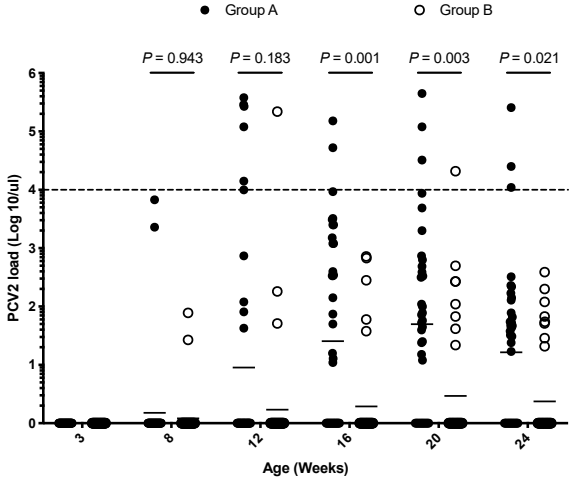

Figure 1. The overall PCV2-viremia over time from both groups. The dash line indicates the threshold for the presence of clinical signs of PCVAD. The long horizontal lines represent the mean concentrations for each group. *P* values less than 0.05 was considered statistically significant difference between groups.

Table 1. Area under the curve of PCV2 load (log10 copies per microliter) in serum samples from 3 to 24 weeks of age in four natural PCV2 infected farms.

Farm	Group	Area under the curve	*P* value
1	A	445.58	0.0001**
	B	48.04	
2	A	155.06	0.988
	B	145.33	
3	A	132.98	0.0001**
	B	0	
4	A	45.28	0.054
	B	9.72	

***P* values less than 0.001 was considered statistically very highly significant difference between groups.

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Improvement of performance, lung health data and reduction of antibiotic use after changing PCV & *M. hyopneumoniae* prophylaxis to Porcilis® PCV M Hyo two-shot vaccination in a fattening farm in North West Germany

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Introduction

Diagnosis of respiratory problems in fatteners is often challenging, especially when several different pathogens are involved. In swine dense areas like in North-West Germany combined infections with PCV2, *M. hyopneumoniae*, PRRS and *APP* are not unusual in growing pigs. The goal of this investigation was to stabilize the critical clinical situation on a farm by adapting the current vaccination protocol.

Materials and Methods

Beginning in 2018 severe clinical respiratory signs appeared in a typical fattening farm (1-to-1, 2400 animals) in North-West Germany. The piglets were vaccinated with a PCV & *M. hyopneumoniae* freshly mixed vaccine and PRRS EU strain. Results of diagnostic examinations (necropsies, blood samples) showed a low virus load of PRRSV and PCV2. *M. hyopneumoniae* was detected in lung samples including related histological damages. Serologically, *APP* serotype 12 antibodies were detected. A slaughterhouse-check of lungs showed 100% of lungs affected by pneumonia (43.5 % severe) and more than 15 % with mild to severe pleuritis. After these findings the PCV & M. hyo vaccination protocol was changed to Porcilis® PCV M Hyo 2-shot (the vaccine can be used in a one or two shot regimen; here 1 ml day 3, 1 ml at day 21) as a first step to stabilize the respiratory situation. This vaccination regimen is expected to support a strong M. hyo protection and to avoid the door opener function M. hyo may have on other pathogens (for instance APP).

Results

After changing the vaccination protocol, the clinical situation of the respiratory problems was immediately stabilized. Losses were reduced from 7.47 % to 2.31 %, average daily weight gain (ADWG) increased from 857.6 to 922.4 g/day within the next 3 months (Table 1). The consumption of antibiotics has been reduced by 77.6%, the respiratory related antibiotic treatments by 93.6%. Slaughterhouse-checks (Figure 1) of the first groups after vaccination switch showed a decrease of lungs affected by severe pneumonia to <1 %. Pleuritis (mild – severe) was seen in just 3 % of the lungs. The continuous improvement of lung health was seen also on a further check 5 months later.

Conclusions and Discussion

Noticeable improvements in animal health can be achieved through tightly meshed herd diagnostics and

adaptations of herd- and vaccination management, particularly through adaptations of the vaccination protocol in a complex clinical situation. Besides direct effects on typical M. hyo related lesions also reduction of clinical damages by further bacterial coinfections were seen.

Table 1. Performance data and antibiotic use before and after vaccination change of the fattening pigs.

period	04/21/18-09/28/18	10/19/18 - 01/08/19	+/- %
pigs, n	3352	1599	
vaccination PCV M. hyo	freshly mixed	Porcilis® 2-shot	
losses, %	7.47	2.31	-69.1
ADWG g/d	857.9	922.4	+7.52
antibiotic treatment d/pig	6.83	1.53	-77.6
respiratory antibiotic treatment d/pig	6.22	0.40	-93.6

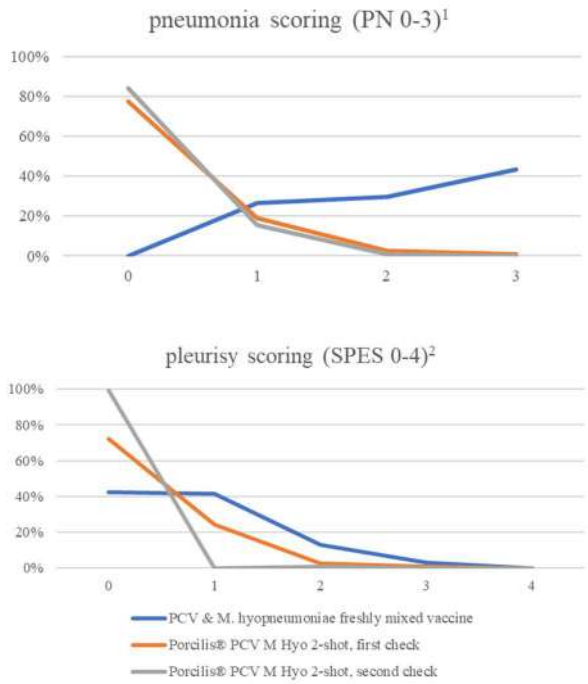

Figure 1. Pneumonia and pleuritis scores results of slaughterhouse checks with different PCV M. hyo vaccination regimen

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Field evaluation of pig growth and mortality indexes of ready-to-use PCV2-*M. hyopneumoniae* vaccines

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Introduction

PCV2 and *Mycoplasma hyopneumoniae* are two of the most common pathogens in swine production, which lead to a reduction in the productive parameters. Vaccination is the main tool to avoid these losses. Provided that a great number of farms include these two agents in their vaccination programmes, in order to improve farm management, ready-to-use vaccines against PCV2 and *M. hyopneumoniae* have been developed.

The aim of this study was to compare the effectiveness of two ready-to-use PCV2-*M. hyopneumoniae* vaccines, between them and with an unvaccinated group, by assessing mortality indexes and measuring the average daily weight gain of the pigs.

Materials and Methods

The study comprised two consecutive production batches in a 200-sow commercial farm where PCV2 Systemic Disease (PCV2-SD) had previously been diagnosed in fattening pigs.

At 4 weeks of age, when weaning took place, all piglets were identified, individually weighed and randomly allocated into three homogenous groups, considering weight and number of deliveries. Two of the groups were vaccinated with a ready-to-use bivalent vaccine against PCV2 and M. *hyopneumoniae,* whereas the third one was used as a control group. Thus, 213 piglets were vaccinated with Porcilis® PCV M Hyo (Group A), 207 piglets were vaccinated with Suvaxyn® Circo+MH RTU (Group B) and the 226 remaining piglets were injected with sterile saline solution (Group C). Each pig was subsequently weighed at 11 weeks old (beginning of the fattening period) and at 23 weeks of age (end of the fattening period).

In each group, the mortality rate was determined for each production phase and a comparison was established between them using the Chi-square test. The Average Daily Weight Gain (ADGW) was assessed throughout the whole life of each pig, as well as throughout the nursery and fattening phases specifically. Subsequently, the average growth was compared among the three groups using an ANOVA.

Results and Discussion

In the vaccinated groups the mortality rate was lower than in the control group, although the difference was not significant ($p > 0.05$).

The ADGW in the nursery phase was 0.373 kg in Group A, 0.383 kg in Group B and 0.369 in Group C, being statistically equal ($p = 0.219$). Throughout the fattening phase, pigs in Group A showed a significantly higher ADWG (average 0.861 kg) compared to those in Group C (average 0.827 kg) ($p = 0.015$). Significant differences in the ADWG were not observed between pigs in Group B and those in each of the other groups ($p > 0.05$).

Overall, the ADWG among the vaccinated pigs was statistically equal. However, only the administration of Porcilis® PCV M Hyo led to a significant increase in the ADWG ($p = 0.036$) regarding the unvaccinated pigs.

Conclusions

Both vaccines seem to reduce the mortality rate of pigs throughout their productive phases. Likewise, both tend to improve the ADWG, but a significant increase of this value in relation to the unvaccinated pigs was only observed when Porcilis® PCV M Hyo was administrated.

Acknowledgments

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Space, time, and genetic relatedness of porcine reproductive and respiratory syndrome virus (PRRSv) in the United States

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Introduction

Molecular epidemiology of PRRSv has become a popular approach during outbreak investigations, surveillance and breeding herd stabilization programs and re-breaks through the sequencing of the envelope protein encoding gene ORF5. Still, little is known regarding the relative contribution of local spread (e.g. pig movement, fomite, or airborne) versus long distance (e.g. long-distance pig movements) factors on viral transmission. Here, we aim to assess the relationship between space-time clustering and genetic diversity of PRRSv ORF5.

Materials and Methods

PRRSv sequences from the Morrison Swine Health Monitoring Project (MSHMP), a national initiative aimed at monitoring swine diseases such as PRRSv representing close to 50% of the U.S. sow population were used in this study. Sequences with 100% nucleotide identity from the same farm up to 30 days apart, and ≥98% nucleotide identity with strain VR2332 (parent strain of the first modified-live vaccine) were excluded from the study. A total of 5,975 sequences from 13 states that were obtained between 2009 − 2019 were included in the analysis. Because sequences were mostly distributed in two U.S. regions, analysis was stratified per region as defined by the U.S. Census. A space-time permutation model was applied to identify space-time clusters during the studied period using SatScan™. To account for the fact that systems might monitor disease differently, and thus generate different amount of data, the swine production system was considered in the clustering analysis. When a cluster was identified, all sequences within the cluster (cases) were compared to all sequences outside clusters but from the same time period (e.g. ± 3 months; controls). Sequences were compared by analysis of molecular variance (AMOVA), and then according to their mean percent nucleotide identity by Kruskal-Wallis rank sum test, and according to RFLP diversity by Shannon diversity index.

Results

Three space-time clusters were found in Region 1 and 25 in Region 2. Clusters ranged from 0.6 to 75.5 km radius with a duration time range between 6 and 286 days and included between 4 and 57 sequences. We found that being a case or a control significantly explains the genetic variability of 17 of these clusters by analysis of molecular variance (AMOVA p-value<0.05). Among those, median percent identity was higher among cases than among controls for 14 of the 17 clusters and lower for the remaining 3 clusters, indicating that on average case sequences are more similar to each other than control sequences are to each other. RFLP Shannon diversity index was higher among controls for all 17 clusters, indicating that on average controls have a higher variability of RFLP patterns than cases.

Conclusions and Discussion

We found that being inside or outside a space-time cluster significantly explains the genetic variability of most, but not all of the cases. The relationship between space, time, and genetic factors in PRRSv transmission is complex and controversial.[1–3] However, we found that overall trends might miss important case by case information. Some limitations need to be acknowledged. First, our space-time cluster identification only accounts for the events that are reported. However, farms or systems that do not sample or participate in the surveillance project may still contribute to local transmission. Additionally, it does not account for other processes involved in transmission such as movement of animals, personnel sharing between farms and any other indirect routes of transmission.

This study highlights the broad range of applicability of data generated by molecular surveillance of PRRSV. Maintaining a comprehensive up to date dataset can potentially also allow for prospective monitoring new regional outbreak occurrences, allowing for timely interventions.

Acknowledgments

The authors would like to acknowledge the Morrison Swine Health Monitoring Project (MSHMP) participants and veterinarians for their collaboration in sharing PRRSV sequence data, as well as the collaborating Veterinary Diagnostics Labs. The project was funded by the Swine Health Information Center (SHIC).

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Molecular characterization of type 2 porcine reproductive and respiratory syndrome virus (PRRSv) in the United States

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Introduction

The Morrison Swine Health Monitoring Project (MSHMP) is a national voluntary initiative that besides working towards foreign disease preparedness it also monitors PRRSv. The project started in 2011 collecting data both retrospective and prospectively, and currently represents close to 50% of the U.S. sow population. Participants not only share sow farm PRRSv status weekly, but also PRRSv ORF5 sequences from their routine monitoring (including breeding herds, growing and finishing sites). Here, we aim to describe PRRSv occurrence in the U.S. based on this molecular surveillance.

Materials and Methods

Data comprising a total of 26,831 PRRSv Type 2 sequences from 2009-2019 was analyzed. We used MAFFT alignment on Geneious Prime to calculate the percent identity (e.g. amount of nucleotides characters that match exactly) between each sequence and the first type 2 PRRSv isolated in the U.S. (VR2332) and its complement (percent nucleotide difference). Sequences were described according to herd type and percent nucleotide difference by the year in which they were submitted for diagnosis. Additionally, sequences were classified into RFLP patterns, except for those that included ambiguities. We also described the frequency of RFLP type 1-7-4 over time within that dataset and the percent nucleotide difference of each sequence to the first 1-7-4 type identified in our data. RFLP type 1-7-4 sequences were also classified into lineages[1] and sublineages[2].

Results

Overall, we observed a median nucleotide divergence of 12.6% (IQR: 11.3 – 14.1) from the reference VR2332 since 2009, although a small percentage of sequences remained less than 5% different from the reference. A total of 52.6% of the sequences contained information on herd type. We found that the distribution of nucleotide distances to VR2332 was different between breeding herds and grow-finish herds (median 12.7% with IQR 12.1% – 14.1% and 12.4% with IQR 8.6% - 13.7%, respectively; Kruskal-Wallis *p*-value=0.0001; Figure 1). We also found that RFLP type 1-7-4 sequences represented less than 1% of our data up to 2013; however, it quickly increased to 21% in 2014, peaked at 42% in 2015 and then started to slowly decrease with 41%, 31%, 23%, and 16% in 2016, 2017, 2018, and 2019, respectively. We found that the median and range of percent divergence of all PRRSv RFLP type 1-7-4 from the first type 1-7-4 within MSHMP database has decreased from 3.6% (min: 2.3, max: 13.9) from 2009-2015 to 4.3% (min: 2.5, max: 8.6) on 2016- 2018. It also had a small increase to 4.8% (min: 3.3, max: 10.8) in 2019.

Conclusions and Discussion

It was not possible to see a clear pattern of increasing divergence. However, we found that the range in nucleotide difference varies largely between years. The larger range in percent nucleotide identity compared to VR2332 in growing pigs suggests a higher viral diversity within this group. The decreasing range of PRRSV 1-7-4 percent divergence from VR2332 is consistent with lineage turnover previously described[2].

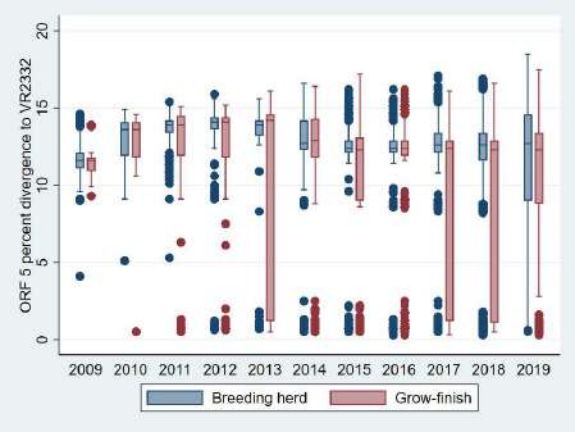

Figure 1. PRRSV ORF5 percent nucleotide difference from VR2332 by year and herd type.

Acknowledgments

The authors would like to acknowledge the Morrison Swine Health Monitoring Project (MSHMP) participants and veterinarians for their collaboration in sharing PRRSV sequence data, as well as the collaborating Veterinary Diagnostics Labs. This project was funded by the Swine Health Information Center (SHIC).

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Serological survey for Influenza A virus in free-living wild boars in Southern Brazil

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Introduction

Influenza A virus (IAV) is members of Orthomyxoviridae family, cause an acute respiratory disease that affects different animal species, including humans (1,3,4). In swine co-infected with different variants of IAV, can occur the process known as genetic rearrangement, leading to the emergence of novel pandemic strains. AIV has been detected in commercial pigs (3,4) and in wild boars (1). Wild boars, including Eurasian wild boars (*Sus scrofa* Linnaeus), feral pigs *(Sus scrofa domesticus*), and hybrids between the two, is an exotic invasive species present in more than 20 Brazilian States (2). Southern Brazil (Paraná (PR), Santa Catarina (SC) and Rio Grande do Sul (RS) States) is the most important region for commercial pigs and wild boars are commonly found these regions (2). These wild populations are highly mobile and have many opportunities to come into contact with pathogens from commercial pig, poultry and humans (1). The aim of this study is to provide a preliminary overview of the circulation of IAV in free-living wild boars subpopulations hunted between the years 2017 and 2019 in the south region of Brazil.

Materials and Methods

Between 2017 and 2019 wild boar sera samples were obtained with the collaboration of hunters licensed for population control of wild boar, in accordance with Brazilian legislation. The hunting location (state and municipality), gender and body weight categorized into juveniles (<30kg) and adults (≥ 30Kg) were recorded by hunters. 456 blood samples were collect immediately after hunting by puncture in the cavernous sinus or heart, by exsanguination (cervical major veins) or from the thoracic cavity, and transported to the laboratory for centrifugation and stored at −20 ° C until serological analysis. The serological screening to detect IAV-specific antibodies were perform using a commercially available ELISA kit (IDEXX AI MultiS-Screen antibody (Ab) test), according the manufacturer's instructions. The prevalences were estimated considering the sensitivity (95,4%) and specificity (99,7%) of the test. For comparison of the prevalence between different States, age groups (body weight) and gender, Fisher's exact test was applied. Differences were considered significant at P ≤ 0.05.

Results

The serology results are summarized in Table 1.

Table 1. Influenza A virus seropositivity in free-living wild boars subpopulations in southern Brazil

State	No. positive /Tested	Prevalence (%)	95% IC
Rio G. do Sul[2]	3/240	1,0	0,0 – 2,3
Santa Catarina[2]	4/111	3,5	0,0 – 6,9
Paraná[1]	24/105	24	15,7 - 32,2
Total	31/456	6,8	4,5 – 9,2

[1]Superscripts indicate statistically significant diferentes ($p \leq 0.05$).

Paraná had a higher soroprevalence for Influenza A, differing from other states ($p<.0001$). There were no difference between age and gender groups ($p> 0.05$).

Conclusions and Discussion

To the knowledge of the authors, this is the first report of IAV in free-living wild boars in Brazil. The prevalence of IAV has been low in wild boars in SC and RS. However, the high seroprevalence for IAV in wild boar in Paraná indicates greater exposure to the virus, which may be associated with the varied interactions of these populations with the environment, including intra and interspecies contacts. These preliminary results indicate the need of research to identify the factors associated with wild boars IAV seroprevalence in different subpopulations and the influenza A virus serotypes that circulate in these populations.

Acknowledgments

We are thankful to the Oficial Veterinary Services from SC. PR and R and to the hunters for help with wild boar samples, especially Rodrigo Menon de Souza, and members from Javali no Pampa group, La Hire Medina and Tiago Xavier.

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Evaluation of shedding and effect on pig performance of prevacent® PRRS vaccine

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Introduction

Many swine production systems today are vaccinating PRRS negative pigs from at risk populations with PRRS vaccine. Vaccination has been shown to improve performance in the face of field virus infections.[1,2,3,4] However, vaccination has reduced post-weaning growth performance in absence of PRRS exposure.[5] Protecting PRRS negative weaned pigs placed in high PRRS risk areas helps reduce shedding of field virus if infection occurs and helps reduce area PRRS spread. The use of PRRS vaccine may be one of these tools. Elanco licensing data for Prevacent® PRRS from a small group of pigs in a research setting showed limited transmission from vaccinates to unvaccinated sentinels.[6]

Objective

Objectives of this study included: (1) identifying the level of viremia and shedding in pigs following vaccination with Prevacent PRRS® vaccine in a commercial setting, and (2) measuring any impact of vaccination on performance of the pigs.

Materials and methods

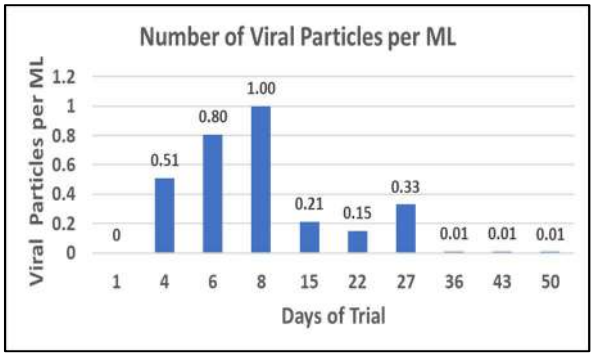
- 30 sentinel pigs in each treatment were identified and tagged in each ear and blood tested at various times in the study.
- Oral fluids were collected from each pen at the same time blood samples were collected.
- Day 0: Prevacent® PRRS vaccine administered at labeled dose (1 ml/pig). Controls administered 1 ml saline.
- Body weights collected at start (day 0) and end (day 50) of nursery period.
- Cyclonic Air collector was used to collect air samples at daily intervals from Day 0- Day 28; samples were collected from inside the barn, at pit fan, and 1 mile away from the site in the downwind direction for the day.

Results

All air samples collected were negative from all locations demonstrating that there was no detectable shedding by this method of sample collection.

Pigs serologically converted to vaccine showing an immune response to the vaccine.

The levels of virus dropped quickly following exposure which may explain that there was limited shedding detected from the population.



Vaccination did not affect nursery growth performance.

Item	Control	Vaccinate	Total
No. Pigs	462	521	983
BW, lb			
Day 0 (kg)	6.5	6.4	6.4
Day 50 (kg)	34.8	34.9	34.9
ADG, (gms)/day	568	572	568

Conclusions and Discussion

Vaccine virus did not shed into the air in this study based upon sampling completed. Viremia decreased quickly after vaccination and may be in part why there was limited spread. Vaccination did not affect nursery growth performance.

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Antigen and antibody of Classical Swine Fever from Korean wild boars: 2016-2019

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Introduction

Classical swine fever virus (CSFV) is a single-stranded RNA virus belonging to the genus pestivirus (family, Flaviviridae). Classical swine fever (CSF) is one of the most important viral diseases affecting domestic pigs and wild boars [1]. Wild boars are as susceptible to CSFV as domestic pigs; therefore, eradication of CSF from wild boars is of epidemiologic value because it can prevent spread among domestic animals [2]. Monitoring of CSF in 5620 Korean wild boars captured between 2010 and 2014 identified only seven animals with CSFV and 23 animals with anti-CSFV antibodies [3]. CSFV (YC16CS strain) isolated from an outbreak in breeding pigs in the north of Gyeonggi in 2016 shows a high genetic similarity and the same sub-genotype (2.1d) as the CSFV (CW17WB) strain isolated from wild boars in 2017 [4]. The risk of CSFV transmission from wild boars to breeding pigs is clear [4]. Therefore, we attempted to identify the reasons underlying the rapid spread of CSF infection among wild boars to guide development of prevention measures.

Materials and Methods

From 2010, wild boars were hunted in co-operation with the Korean Pork Producers Association and the Korean government to satisfy the OIE requirements for surveillance of wild boar and feral pigs in CSF-free countries. Blood samples were collected from 6970 wild boars hunted in nine provinces in South Korea between February 2016 and November 2019. Blood was collected in heparinized tubes. Total RNA was extracted using a micro column-based RNeasy Mini kit (Qiagen, CA, USA). The RT-PCR conditions and specific primers used to amplify the complete E2 gene have been reported previously [4, 5]. Serum neutralization (SN) tests based on a neutralizing peroxidase-linked assay (NPLA) were performed to detect CSFV-specific neutralizing antibodies.

Results

Korean wild boars captured between 2016 and 2019 comprised 40.2% females, 48.2% males, and 11.6% unknown. Of these, 4.7% (128/2799) of females, 3.7% (123/3362) of males, and 2.4% (19/808) of unknown animals were positive for anti-CSFV antibodies. With respect to age, 34.2% (2387/6970) of captured boars were under 1 years old, and 39.8% (2771/6970) were 1–2 years old. In addition, 2.1% (51/2387) of boars under 1 years old, 4.9% (136/2771) aged 1–2 years old, 6.2% (36/583) aged 2–3 years old, 8.6% (22/255) aged 3–4 years old, 4.2% (5/119) aged over 4 years, and 2.8% (24/855) of indeterminate age were positive for anti-CSFV antibodies. The anti-CSFV antibody-positive rates in Gyeonggi (GG) increased continuously over the years: from 1.7% (5/302) in 2016 to 4.7% (6/129) in 2017, 9.3% (9/97) in 2018, and 14.3% (65/453) in 2019, as did the rates in Gangwon (GW) (from 0.7% (1/148) in 2016 to 4.8% (12/251) in 2017, 16.5% (33/200) in 2018, and 21.2% (129/608) in 2019)

Conclusions and Discussion

The rapid increase in CSF rates in wild boars may be due to a continuously circulating infection within hub area and increased population density. The main area of CFSV-positive wild boars may be shifted by external factors such as hunting; this may play an important role in spreading the disease.

Acknowledgments

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Detection of PCV2 and PCV3 in flies from pig farms in Brazil

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Introduction

Porcine circovirus (PCV) is responsible for economic losses in swine production. Vectors such as flies (*Musca domestica* and *Culex* sp) are considered one of the most important disease carriers (1, 2) in which circovirus could be detected. (3, 4). The PCV3 is considered an emerging pathogen with a high prevalence, but there are few studies showing transmission routes mainly related to invertebrates, which has already been related for PCV2 virus (5). The aim of this study was to investigate the presence of PCV2 and PCV3 in flies, and its roles in transmission of PCV2 and PCV3.

Material and methods

The flies *Musca domestica* (n = 11) and *Cochliomyia hominivorax* (n = 24) species were collected from a positive farm for PCV2 and PCV3 located in the Minas Gerais State, Brazil. Samples were collected at nursery pens at different ages (21 to 70 days).

Total DNA from these mosquitoes were extracted and then tested for PCV2 nucleic acid by PCR using a commercial kit (Wizard® Genomic DNA Purification Kit - Promega) and the PCR was performed following protocols previously described adapted from Kwon *et al*., 2017 (5).

Results and discussion

All the samples from *Musca domestica* tested were positive for the PCV2 virus and 10 of 11 samples were positive for PCV3 (Table 1). For *Cochliomyia hominivorax* 20 were positive for PCV2 and 16 were positive for PCV3 (Table 1) showing the presence of these viruses in insects. This study demonstrates that these insects could serve as the potential vector during the transmission of PCV2 as demonstrated in previous studies for other viruses in swine (1,6). In addition, insects collected from swine farms can be a tool for health surveillance and measurement of virus circulation due to the easy capturing without animal manipulation.

Table 1: Number of positive results for PCV2 e PCV3 stratified by species.

	PCV2 positive	PCV3 positive
Musca domestica	11 (n=11)	10 (n=11)
Cochliomyia hominivorax	20 (n=24)	16 (n=24)
Total	31 (n=35)	25 (n=35)

Another important information to be discussed is the capacity to spread this pathogen to other pens and farms. In general, *Musca domestica* has the capacity to travel large distances, which may difficult programs of control and eradication in high-density animal regions.

Therefore, the real role in the epidemiology of illnesses caused by Circovirus should be investigate in order to create strategies to minimize virus spread via insects.

Conclusion

The PCR test for detection of PCV2 and PCV3 on flies was efficient to detect the virus and can be an important tool for surveillance in swine farms. The positivity in flies is considered a risk due to fly ability to carry these pathogens to other farms, which may difficult control and eradication programs.

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Porcine circovirus 3 in wild boars (*Sus scrofa scrofa*) in Rio Grande do Sul State, Brazil

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Introduction

Harboring a genome more related to bat and canine circovirus, porcine circovirus 3 (PCV3) was first described in 2016 (3) and has been detected in pigs in several countries, including Brazil (6, 7). Ever since PCV3 has been associated with several pathological disorders as stillbirths (5) and cardiac and multi-systemic inflammation (3), but also mostly found in healthy animals compared to unhealthy pigs (5). Additionally, wild boars (*Sus scrofa scrofa*) are susceptible to several pathogens, including PCV3 (4). Consequently, the potential risk of pathogen transmission between the wild boars' population and domestic pigs must be hardly considered. Here, we described the PCV3 occurrence in wild boars in the Rio Grande do Sul State, Brazil.

Materials and Methods

Twenty-six wild boars (10 females and 16 males) were slaughtered through the official program of wild boards population control, in RS, Brazil. DNA from 180 tissue samples of lung, liver, spleen, kidney, tonsils, heart and lymph nodes was extracted using a commercial kit, following the manufacturer's instructions. Conventional PCR amplification was used for the detection of PCV3 (2) and PCV2 (5) as previously reported.

Results

PCV3 was detected in 15.4% (4/26) of the animals sampled. For PCV2 we found 57.7% of positivity. Co-infection with PCV3 and PCV2 was detected in one animal (from spleen sample). The DNA of these two circoviruses were found in several tissues (Table 1).

Conclusions and Discussion

This study reports the first detection of PCV3 in wild boars from Brazil and a co-infection with PCV2. The findings suggest that PCV3 has been circulating in the South region of Brazil, where the majority of the industrial swine is performed. Direct PCR proved to be very sensitive for circoviruses screening, especially from lung, heart, kidney and tonsils tissue (Table 1). Recently, PCV3a and PCV3b genotypes were reported in German (4). Thus, consdering the existence of PCV3 variants, the PCV3 found were submited to sequncing in order to deterrmine their genotypes.

Based on the results reported here, PCV3 has a tropism for a high range of cell types, which are susceptible to infection. These findings are in agreement with PCV3 ability to affect different anatomical sites, causing reproductive failure, encephalitis and myocarditis in perinatal piglets, porcine dermatitis and nephropathy syndrome, and periarteritis in swine (1).

Although we found 3.8% (1/26) of PCV2 and PCV3 co-infection, further studies are necessary to state whether there is interference or not in each other epidemiology and immunopathogenesis. As previously reported, PCV3 circulation has no impact on PCV2 vaccine efficacy, as well as the intensive PCV2 presence in non-vaccinated farms did not seem to increase the level of PCV3 infection (8). However, once the available vaccines have not cross-protection against heterologous circovirus, the occurrence of PCV3 in wild boars may be a challenge to commercial pig production.

Table 1. Percentage of positivity for circovirus according to the tissue analyzed.

Organ	PCV2	PCV3
Heart	23.1	3.8
Kidney	26.9	3.8
Liver	-	3.8
Lung	23.1	7.7
Lymph nodes	7.7	-
Spleen	-	3.8
Tonsil	15.4	7.7

It is common knowledge that we are facing the emergence of new pathogens and genotypes with high potential to cause animal and human diseases. Therefore, we reinforce the importance of epidemiologic screening of wild boars in Brazil and worldwide considering its impact on public health and economic losses.

Acknowledgments

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Controlling porcine reproductive and respiratory syndrome virus in a 15,000-sow herd: Is the load-close-expose protocol still effective in very large sow populations?

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Introduction

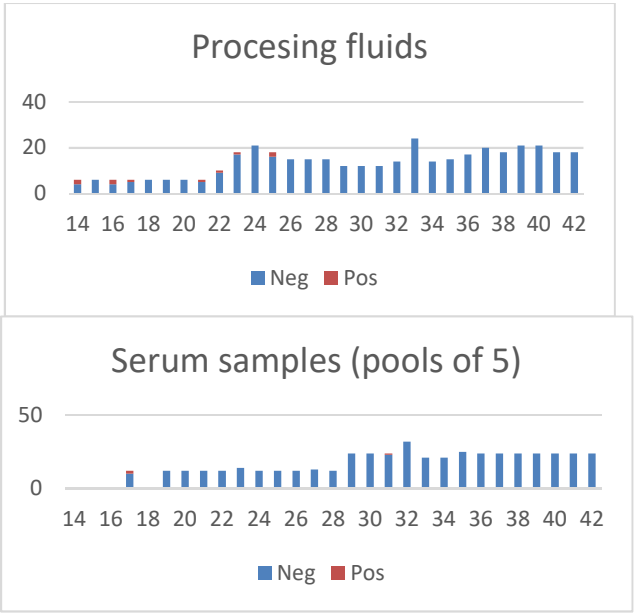
Porcine reproductive and respiratory syndrome virus (PRRSv) is one of the most important viral diseases of swine. Economic losses in the swine industry due to PRRSv infection are associated to reproductive failure, increased mortality and respiratory disease. The goal to control and eliminate PRRSv virus from the sow herd is first to eliminate virus transmission, then stabilize the herd (consistently wean negative pigs) and finally recover the negative status of the population. Multiple protocols have been developed to achieve this target; which may include live virus inoculation (LVI) or modified live virus (MLV) or live attenuated vaccines. Nevertheless, herd closure and strict control of piglet movements in the farrowing rooms is crucial to eliminate the virus whether LVI or MLV is used. The effect of herd closure after LVI in large sow farms is not completely understood. Hence, the objective of this study was to evaluate the time required for stabilization after a load-close-expose protocol using LVI in a 15,000-sow herd in Mexico.

Materials and Methods

In November 2018 PRRSv was first detected by PCR in a 15,000-sow herd previously negative to PRRSv, *Mycoplasma hyopneumoniae* and porcine coronaviruses. LVI was performed intramuscularly in site one during the second week (W2) of 2019. Herd closure was initiated on W3 2019, at which time a batch of naïve gilts was also exposed to the same LVI in the quarantine site of the sow herd. Gilts in the quarantine were moved to site 1 between W7 and W11 2019. Strict biocontainment protocols were established in the farrowing rooms to avoid PRRS virus transmission within the sow herd and all piglets were weaned off site during the stabilization program. Six to 21 processing fluids (PF) were collected on a weekly basis between 14 and 42 weeks after LVI. Additionally, 60 to 160 serum samples (SS) of piglets at weaning were collected on a weekly basis between 19 and 42 weeks after LVI. PF and SS were tested individually and in pools of 5 respectively for PRRSv by PCR. Tabular methods were used to estimate the percent of positive samples over time. Additionally, the proportion of positive and negative samples within a 29-week follow up period between PF and SS was considered statistically different if the p value for the McNemar's test was lower than 0.05. Finally, the time to stabilization (consistently wean negative pigs) was established based on the number of consecutive weeks weaning pigs negative by PCR in PF and SS.

Results

Ten out of 396 PF (2.5%) and 3 out of 474 pools of SS (0.6%) tested positive for PRRSv by PCR (Figure 1) within a 29-week follow-up period (W14 to W42 after exposure). The percent of PF by week ranged between 0 and 33.3% while the percent of positive pools of SS by week ranged between 0 and 16.6%. Within the 29-week follow up period the odds of a positive PF was 3.9 times higher than the odds of a positive pool of SS. However, the last week at which PF's were found positive was 25 weeks after LVI while the last week SS pools were found positive was 34 weeks after LVI. In this population, the time to stabilization was 32 weeks after LVI at which PF and SS pools started testing negative consecutively for 10 additional weeks (Figure 1).



Figuare 1. Number of negative and positive samples by week after LVI.

Conclusion

Our results indicate that LVI and herd closure is still an effective method to stabilize PRRS virus transmission in sow herds even when the population is as large as 15,000 sows in the same site. Furthermore, the time to stability was within the expected range reported in other studies. However, larger sow populations represent different challenges in terms of biocontainment and sampling strategies. Moreover, our result supports other research in which PRRSv is more likely to be found in processing fluids than in serum samples at weaning during herd closure. However, caution should be taken because the latest positive sample in our study was from serum samples at weaning, and not from PF, indicating that at very low prevalence the virus might not be found in processing fluids when is still circulating in the population.

Populating a PRRSV and PEDV negative breeding herd from a positive source

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Introduction

The decision was made to populate a new farm in Northwest China from a PRRSV and PEDV positive multiplication herd and to generate a PRRSV and PEDV negative herd after the stocking. The source farm is farrow to finish, with on-site gilt development unit (GDU) and boar stud.

Materials and methods

Disease status of the source farm

The multiplication herd was vaccinated with PRRSV MLV but vaccination ceased in March 2019. PRRSV was tested monthly, and on gilts for client deliveries. PRRSV PCR positive samples were detected in a routine surveillance in May 2019 and a new strain was identified compared with the strain identified in December 2017 in this farm.

A PEDV outbreak occurred at the source in February 2019. Whole herd exposure via feedback was implemented, and a bubble without pigs in the farrowing room was created for two weeks to allow the newly born piglets to develop strong immunity and to avoid persistent circulation of virus in the sow herds and shedding of virus to piglets.

Sow and boar selection

One month before movement, introduction of replacement gilts from G/F barns was stopped.

It was decided that boars older than 300 days and sows after parity one and with over 40 days to farrowing should be selected, but transportation of 2,000 sows would take 16 days to complete, so finally sows over 56 days to farrowing were selected for the new farm. Sows were thoroughly washed and disinfected with Virkon 1% prior to loading.

Transport biosecurity

Transport vehicles were externally sourced from a third-party fleet and tested for ASFV before admission. Then the vehicles were thoroughly washed, disinfected, and baked at 56 for 70 minutes at a dedicated truck wash facility. All truck washers and drivers were trained to comply with biosecurity standards.

Receiving farm preparation

The farm is a fenced farm with air filtration and is bird proof. It was completely washed, foamed, washed again, then disinfected with 2% Virkon, fumigated with formaldehyde, and closed for three days before stocking.

Measures adopted in the farrowing rooms prior to confirmation of PRRSV negative status

Biosecurity was observed and protocols were strictly implemented at the farm to avoid introduction of diseases via contaminated fomites or visitors. No fostering was allowed in the farrowing rooms, and weak piglets were euthanized.

PRRSV and PEDV surveillance after stocking

When the first litters of piglets born reached 5 weeks of age for three consecutive weeks, 15-30 serum samples and 5-15 rectal swab samples were collected from 3, 4 and 5 week old piglets. When litters reached 10 weeks old, 10 serum samples were collected from 6, 8 and 10 week old piglets. Serum samples were tested with PCR for presence of virus and ELISA (IDEXX) for antibodies. Fecal swabs were tested with PCR for presence of PEDV.

Results

All serum samples were PRRSV PCR negative and rectal swab samples were PEDV PCR negative. Serum sample results also indicated the lack of PRRS antibodies. One sample indicated a suspect positive S/P ratio for PRRSV antibody, but PCR result was negative, and a sample from this pig submitted 10 days later indicated PRRSV antibody negative.

Conclusion and discussion

A PRRSV and PEDV negative herd was generated from a positive source by creating a farrowing room bubble and selecting individuals that had been infected several months before the movement. Continuous surveillance and strict biosecurity to prevent introduction of diseases are critical to maintain the high health status.

Acknowledgement

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A case study: use test and removal strategy to contain an ASFV outbreak in a farm

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Introduction

Since being detected and reported in China on August 04, 2018, ASFV has spread across the country and become endemic. The impacts of this disease are enormous, and it continues to challenge the veterinarians, and the pig production industry because of heightened risks.

This abstract distills the test removal strategy and how it succeeded in containing the ASF outbreak in a case in China in the situations as described.

Materials and methods

Following upgrade in biosecurity infrastructure, the farm under discussion, was used as a temporary isolation facility for incoming gilts. It has five barns and each barn has 12 pens of 60 m^2 /pen (house 40-45 pigs), with a total capacity for 2,800 finisher pigs and serves as a quarantine for a large sow production compound 300 km away.

Figure 1. Layout of farm and inside

The transport of gilts and ASFV detection The transport of 1,400 gilts to this facility by 11 trucks started on Sep 8th and was completed on Sep 20th. 10 out of 11 contracted trucks were from a third party, and tested for ASFV by PCR before admission, they were then thoroughly washed, disinfected, and baked at 56 for 70 minutes at a dedicated truck washing facility. All truck washers and drivers were trained to observe and comply with biosecurity standards.

At day 13 DPA (13 days post arrival), 1 pig was found dead in pen 4 (fig 2), and a couple of pigs were seen showing signs of reduced appetite and less feed intake. They were all from one truckload of 70 pigs. The nasal swabs, fecal swabs, and inguinal lymph node samples were collected from this dead pig.

At 14 DPA, the nasal swabs were collected from each pig in the pens 3, 4 and 5; oral fluids were collected from each pen every day in the whole facility, samples from personnel, supplies and equipment, environmental samples were infrequently sampled and sent to a company owned lab for ASFV PCR testing and results were available within 24 to 48 hours. After 21 days, the oral fluids from each pen were collected every 3 days for ASFV testing.

Removal and decontamination strategy On 14 DPA, the ASFV positive pigs were humanely euthanized (electrocution), and packed in impermeable plastic bags, and moved out of farm to incinerate, with the hallway sealed with impermeable plastics. In the evening of 14 DPA, the left half of barn (pens 1-6) was evacuated, with hallway being sealed with plastics and the door sealed to protect the right half of barn. After completing the evacuation, the left side and hallway was covered with caustic soda, and the whole part being fumigated. Workers for each barn stayed inside the barn to perform the duties. Internal and external biosecurity was strictly reviewed and implemented to prevent further contamination.

Results

On 14 DPA, six nasal swab samples from pen 4 (4 positives (3+)), 3 (1+) and 5(1+) in barn 1 were tested ASFV positive by PCR. There were no further positives detected after 14 DPA. There were no further positives detected after 14 DPA. The remainder of the gilts stayed in the facility for further quarantine and after about 55 days were moved into the sow herds. The evacuation of 250 pigs in 6 pens in the left side of barn 1 represented 17.86% loss of the total population in the facility.

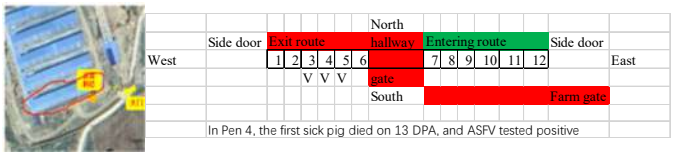

Fig ure 2. ASFV detection, progression and removal map

Conclusion and discussion

If early detection and rapid and biosecure responses are assured, ASFV can be contained in a barn or facility using the test-removal subject to local regulations. It is critical to avoid missing positive pigs and prevent contamination and spread. Risks, especially from transport need to be considered as a potential source of introduction.

Vertical transmission of porcine circovirus type 3 (PCV3) and the effect on piglets Case report in Colombia

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Introduction

Porcine circovirus type 3 (PCV3) is a member of the *circoviridae* family and is associated with a variety of clinical presentations such as dermatitis and nephopathy (PDNS), reproductive failure (1), vasculitis, myocarditis (2), pneumonia (3) and congenital tremor (4). Although it has also been reported in clinically healthy pigs (5). In Colombia, the virus was first described in 2019 (6). The objective of this report is to demonstrate the vertical transmission of PCV3 in gilt and the involvement of the virus as cause of reproductive failure and runt piglets.

Materials and Methods

In June 2019, a farm located in central-western Colombia reported an increase in reproductive failures represented by a reduction in the number of litters, an increase in abortions and mummification. 50 serum samples were taken between gilts and sows of different parities and the presence of PCV2, PCV3, PPV1, PPV7, APPV y PRRSV was determined. Six sows and four gilts were positive only for PCV3 and within these a gilt was chosen that presented a viral load of $7.4x10^3$ (5.8 log genomic copies (lgc)/mL) in serum at the time of delivery and presented a clinical picture of dystocia, a mummy, a litter of ten piglets including two weak-born piglets that died a few hours later. Additionally, one piglet died at week three of age and another one was sacrificed two weeks later. These animals were runts.

The presence of PCV3 was studied in that gilt by qPCR (4) in prepartum, postpartum serum, nasal and vaginal swabs, placenta and colostrum. Likewise, samples of pre and postcalostral piglet serum were evaluated until six weeks of age. Also, mummy tissues and different organs of dead piglets were studied. A complete sequencing of the PCV3 genome isolated from the mummy was done. A histopathological study was also conducted.

Results

The presence of PCV3 in the gilt sampled was verified and different viral loads were detected, being the highest in the placenta (7.23 lgc/mL) followed by prepartum and postpartum serum. A viral load of 10^9 (11.8 lgc/mL) was found also in the mummy. Highest viral loads were also detected in dead piglets. In lung (9,81 lgc/mL) and lymph nodes (6.64 lgc/mL). The vertical transmission of PCV3 was demonstrated by the presence of a viral load of 10^6 (7.93 lgc/mL) in precalostral serum which was gradually decreasing until week six of age reaching 10^3 (4.93 lgc/mL). The most relevant histopathological results were found in the placenta with the presence of severe generalized congestion, microtrombosis and mild mononuclear inflammatory infiltrate. Severe lymphoid depletion and proliferation of reticulohistiocytic cells were found in lymph nodes and spleen. Complete sequencing of the isolated virus showed that it was classified as PCV3c.

Conclusions and Discussion

The association of PCV3 as a cause of disease is a matter of extensive discussion worldwide. In our case, we reported a gilt that presented vertical transmission of the virus. A high tropism of the virus through the placenta would explain the involvement of PCV3 in reproductive failure. Apparently, this effect would be intimately linked to the viral load of the gilt during pregnancy and particularly at the time of delivery. A viral load of 10^3 in the serum of the gilt may led to a weak-born piglets and low litter weight. In piglets, a viral load at birth above 10^6 could be associated with runt piglets and death, as occurs with PCV2 (7). The possible neutralizing effect towards the virus by passive immunity was suggested since the viral load in the piglets was gradually decreasing until six weeks of age. The classification of the isolate found in this study as PCV3c is similar with previous reports where PCV3b and PCV3c were associated with cases of reproductive failure.

Acknowledgments

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Detection and molecular characterization of porcine circovirus type 3 (PCV3) in different regions of Colombia

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Introduction

The circovirus genus was classified into three types: PCV1, PCV2 and PCV3 (1). Phylogenetic analyzes have been used to determine the evolution of PCV3. Different classifications of viral strains have been proposed. One of them classifies PCV3 into three groups called PCV3a, PCV3b and PCV3c based on the amino acid sequence of Cap protein (2). Another classification divides it into two major groups called "a" and "b", this based on the amino acid motifs in Rep, Cap and ORF3 protein (3). The purpose of this study was to determine the prevalence of PCV3 in Colombia and to characterize phylogenetically the strains isolated between 2015 and 2019.

Materials and Methods

We evaluated 759 DNA extractions from pig feces samples from farms in the 32 regions of Colombia that were taken in 2015 to determine the presence of PEDV. Additionally, between 2018 and 2019, 108 serum and tissue samples were taken at random from 19 abortions and stillborns from five regions. DNA extraction was done and the presence of PCV3 was detected by PCR (4). Subsequently, complete sequencing of the viral genome of nine isolates was performed (5). For the analyzes, the nine complete sequences and the nine sequences of the cap gene were compared and aligned with 41 complete genome sequences reported in the GenBank.

Results

The detection of PCV3 by PCR showed a 7.44% (66/886) positive in the total samples. This percentage was higher for the years 2018 and 2019 in tissue samples with 52.6% (10/19), followed by serum with 43.5% (47/108). In the latter case, PCV3 was found at weeks 2, 3, 7, 11, 15, 19 and 23 in the growing and finishing pigs. In feces (year 2015), the detection was 1.18% (9/759). For 2015, 18.75% (6/32) of the regions presented the virus, while for 2018-2019 in 100% (5/5) of the regions were PCV3 positive. From the 56 positive samples, nine complete sequences isolates from seven different regions was achieved. Multiple alignment between the nine complete sequences showed a nucleotide similarity of 99.14% to 99.9% between them. For ORF2, the nucleotide similarity was 98.8-100% and for amino acids 98.3-100%. Compared to reference strains, the nucleotide similarity of the nine isolates was 97.7-99.74% and 97.7-100% for the complete genome and ORF2 respectively. A cap-gene based phylogenetic analysis of PCV3 showed that the nine isolates were located in two clades: PCV3b (3/9) and PCV3c (6/9) according to amino acid mutations (A24/V and R27/K).

Conclusions and Discussion

In this study, the presence of PCV3 in Colombia was determined from the year 2015 in at least nine of the 32 provinces. The prevalence of PCV3 worldwide is variable between 5-65% (5.6). In this study, a prevalence of 7% was found; however, this varied depending on the year, regions and sample type examined, with a higher prevalence for 2019. The analyzes on the cap amino acid motifs of the nine Colombian isolates showed mutations at the level of A24/V and R27/K. The presence of these mutations are the proposals for the classification of PCV3 in three clades: PCV3a, PCV3b and PCV3c. Thus, 33.3% (3/9) of our isolates were classified as PCV3c (two isolates from the same region) and 66.6% as PCV3b. This result is interesting since it has been proposed that PCV3b and PCV3c are associated with cases of reproductive failure. In conclusion, the national distribution of PCV3 in Colombia has been established since 2015 including the border regions and in the main pig rearing areas of the country. Phylogenetic analyzes classify Colombian strains within clades PCV3b and c.

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First report of porcine parvovirus 7 (PPV7) in South America and viral capsid modeling

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Introduction

Parvoviruses are members of the *Parvoviridae* family and are very small non-enveloped viruses that contain the ssDNA genome (1). In 2016, a new parvovirus was discovered in the USA from pig swabs rectal samples and classified into a new genus called *Chapparvovirus* as and was given the name porcine parvovirus 7 (PPV7) (2). The PPV7 has been reported in other countries, such as China (3), Poland (4), and Korea (5). To date, PPV7 has not been reported in any country in South America. The clinical signs specifically caused by PPV7 have not been determined and there are no studies establishing its pathogenicity; although some approaches indicate that it may be important in coinfection with other viral agents such as PCV2 and PCV3 (6). From the point of view of the genome, it is known that PPV7 strains show a greater identity in the NS1 gene while the major differences are found at the level of the cap gene, where additions ranging from 3 to 15 nucleotides can be found (7). In the present study, we describe the first report of PPV7 in South America. The partial genome of the isolates is compared and the capsid structure of the viruses found is modeled.

Materials and Methods

Parallel to the detection of PCV3, we evaluated the presence of PPV7 in 764 DNA extractions from pig feces samples taken from farms in the 32 provinces of Colombia in 2015 to determine the presence of PEDV. Additionally, 126 random sera were analyzed from five provinces between 2018 and 2019. PPV7 was searched by conventional PCR to amplify a 241 bp segment of the VP gene using specific primers as reported (3). The partial genome (90% of the genome) of four isolates of PPV7 was amplified using four sets of specific primers and then sequenced. Sequence data of the isolates were assembled and edited using SeqMan Pro. Multiple sequence alignment was done with CLUSTAL algorithm. The best substitution method was calculated and a phylogenetic construction with a Maximum likelihood analysis was performed using MEGA™ 7. Amino acid sequences from Capsid were modelled using I-TASSER. In addition, ModRefiner was employed to refine the obtained models. Then they were analyzed using the bioinformatics tools ProSA-Web, the Ramachandran plots (RAMPAGE) and TM-Align. Capsid protein surface analysis was performed using the RIVEM (Radial Interpretation of Viral Electron density Maps).

Results

Of the total samples analyzed between 2015 and 2019, 7.9% (71/890) was positive for PPV7, discriminated in 5.76% (44/764) in feces and 21% (26/126) in sera. Strains of PPV7 were found on 13/32 in some Colombian regions. The four partial sequences of PPV7 demonstrated that the isolated viruses were distributed in two different clades according to the presence or absence of insertions in the cap gene. Two strains called Antioquia and Risaralda-PPV7 have an insertion of 5 amino acids in comparison to another strain called Cundinamarca-PPV7 and other reported variants. The phylogenetic analysis of PPV-7 Capsid protein gen by Maximum Likelihood method exhibited two well differentiated evolutionary related groups. One group that possesses the insertion and the other that lacks this insertion, suggesting that sequences either with or without the insertion may circulate worldwide. To all models, amino acids in energetically favourable regions were higher than 95%. There were a different folding when the insertion was present.

Conclusions and Discussion

This is the first report of PPV7 in South America from swine farm samples. Sequenced isolates showed the presence of two groups of PPV7: one with the addition of 15 nucleotides in the cap gene and one that did not have it. This difference has a significant effect when modeling the capsid protein leading to differences in its folding. The effect of this difference has not yet been established, but it probably influences the pathogenesis of PPV7 and the ability to infect different types of cells and therefore a possible clinical presentation.

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Transmission of an influenza A virus with human seasonal H3 in pigs resulted in adaptive mutations in the HA gene

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Introduction

Interspecies transmission of influenza A viruses (IAV) from humans to pigs is relatively common and has led to many human-origin influenza viruses, or gene segments therein, becoming endemic in pigs, including the triple reassortant internal gene (TRIG) constellation that is widespread in North American pigs (1). These human-to-swine spillover events have significantly affected the epidemiology of IAV in pigs. Interestingly, experimental infection of pigs with human viruses often results in low replication and rare transmission among animals (2). Little is known about the evolutionary processes that happen at the human-swine interface that allow for viruses to adapt and efficiently replicate and transmit within the new host. The objective of this study was to evaluate the evolutionary dynamics of a human IAV during replication and transmission in pigs.

Materials and Methods

Since wholly human H3N2 viruses do not typically replicate efficiently in pigs in an experimental setting, we generated a reassortant virus (2HA/NAVic11:6TRIG) containing human seasonal HA and NA genes with TRIG backbone and the 2009 pandemic lineage matrix gene (the internal gene constellation that predominates in North American swine). The human seasonal H3N2 surface genes were from A/Victoria/361/2011 (VIC11). The replication of the reassortant virus was compared to the two parental viruses: a typical triple reassortant swine-origin strain (A/turkey/Ohio/ 313053/2004; OH04) and the wholly human strain (VIC11) in a porcine jejunal epithelial cell line (SD-PJEC)(3).

Four-week-old, cross-bred naïve pigs (n=10/group) were challenged with the reassortant virus (2HA/NAVic11:6TRIG) or the two parental strains (VIC11 or OH04). At 2 days post infection (dpi), 5 age-matched, naïve pigs were placed in the same room as each group, without direct contact, to evaluate aerosol transmission. Nasal swab samples were collected at 1, 3, and 5 dpi and at 5, 7, and 9 days post-contact (dpc) from contact pigs (respiratory). Swab samples were submitted to next generation sequencing (NGS) using a high-throughput Illumina MiSeq sequencing platform to evaluate virus evolution.

Results

Our results show that the reassortant virus (2HA/NAVic11:6TRIG) replicated more efficiently than the whole virus VIC11 in swine epithelial cells, but did not reach the titers of OH04 (Fig. 1A). However, the reassortant virus resulted in replication and transmission in pigs (Fig. 1B), indicating that the TRIG constellation favors replication and transmission of human IAV surface genes in pigs.

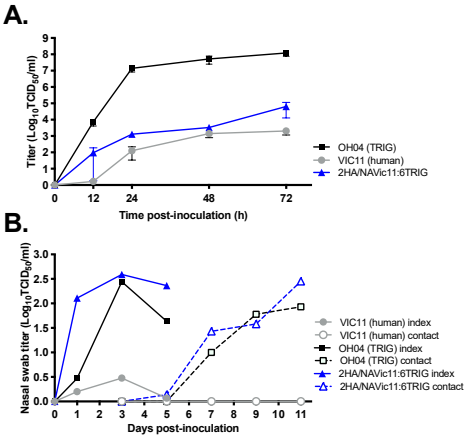

Figure 1. The reassortant VIC11 2:6 OH04 virus replicated in swine cells (SD-PJEC) (A) and transmitted to respiratory contacts (B).

Although the HA gene sequence consensus of the reassortant 2HA/NAVic11:6TRIG) remained invariant in the majority of directly inoculated animals throughout infection, a minor variant in the HA (A154S) was observed at 3 dpi in two animals and became fixed in one animal at 5 dpi. This mutation was fixed in respiratory contact pigs starting at 5 dpc.

Conclusions and Discussion

Determining the evolutionary basis of cross-species transmission is key to understanding the mechanisms that control the emergence of new influenza viruses. Our results show that minor sequence variants in the H3 gene were selected quickly after replication and transmission of human HA in pigs. Further studies are needed to test if the fixed mutation results in increased fitness for the human HA in pigs.

Acknowledgments

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Predominance of rotavirus species B and C in diarrhea outbreaks in suckling piglets from pig herds routinely vaccinated for rotavirus species A

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Introduction

Rotavirus (RV) is considered one of the most important viral pathogens in the etiology of severe neonatal diarrhea in pigs, causing significant economic losses to the swine industry (8,9). The genus *Rotavirus* belongs to the family *Reoviridae* and is classified into nine species (A-I). RVA, RVB, RVC, RVE, and RVH are the species of RVs that affect pigs, of these, RVA is known as the most prevalent in neonatal diarrhea outbreaks (6,8,10). However, among RVs species, the clinical manifestations caused by RVB and RVC have become increasingly frequent and may occur in singular infections or associates with other species of the genus. This study aims to evaluate the frequency of detection of rotavirus in piglets with neonatal diarrhea in Brazilian pig farms.

Materials and Methods

Ninety-three fecal samples from piglets with neonatal diarrhea were evaluated, the age of the animals varied between 1 and 21 days of age. Diarrheic fecal samples were collected between January 2018 and December 2019. The farms were located in the South (n = 11 farms and 46 feces) and Midwest (n = 9 farms and 47 feces) regions of Brazil, totalizing 20 pig farms. Most of the farms were regularly vaccinated with the attenuated commercial vaccine containing the RVA OSU (G5P[7]) and Gottfried (G4P[6]) strains.

The nucleic acid from the fecal samples was extracted using a combination of phenol/chloroform/isoamyl alcohol (25:24:1) and silica/guanidinium isothiocyanate nucleic acid extraction methods (2). Then, the nucleic acid was subjected to electrophoresis test on a silver-stained polyacrylamide gel (ss-PAGE) to observe the electrophoretic migration and confirmed by RT-PCR for RVA (3, 4), RVC (1), and RVH (9). For RVB the samples were tested by semi-nested RT-PCR (5).

Results

Fifty-seven (61.3%) of the 93 diarrheic fecal samples analyzed were positive for RVs. Among the RV species evaluated, were obtained 8/93 (8.6%), 26/93 (27.9%) and 22/93 (23.6%) positive samples in singular infections for RVA, RVB, and RVC, respectively. One fecal sample (1.1%) was found co-infection of RVB and RVH (Table 1). RVH was not found in single infection. Regarding the farms evaluated 3/20, 6/20, and 5/20 were positive for RVA, RVB, and RVC, respectively. Coinfections were observed in two pig farms, one mixed of RVB and RVH, and other mixed infections of RVA and RVC. The other 4 farms analyzed were negatives for all RVs.

Table 1. Single and co-infections detection of rotavirus A, B, C, and H in diarrheic feces of piglets with neonatal diarrhea from pig farms located in the South and Midwest regions.

Regions	Fecal samples RV positives				Negative	Total
	Single infection			Coinfection		
	A	B	C	B+H		
South	8	2	14	-	22	46
Midwest	-	24	8	1	14	47
Total	8	26	22	1	36	93

Conclusions and Discussion

In this study, high rates of detection of RVB and RVC were observed in single infections from piglets with neonatal diarrhea. These rates of detection of RVB and RVC were higher than other studies previously carried out in Brazil (7, 9), in which RVA rates were higher when compared to the detection of RVC and mainly RVB. This decrease in RVA detection is probably due to the widespread use, by Brazilian pig farms, of commercial inactivated vaccines available only for RVA species. The vaccine pressure exerted, possibly favors the increase of diarrheal outbreaks caused by species that until then were not as significant, such as RVB. Finally, these findings demonstrate the importance of different species of RVs in the etiology of swine neonatal diarrhea, until now little remembered, also emphasizing the need for preventive measures to be adopted by pig farms, in an attempt to reduce losses.

Acknowledgments

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First detection of BVDV 1 in free-living wild boars in Brazil

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Introduction

Bovine viral diarrhea virus (BVDV) is a positive-sense RNA virus belongs to the genus *Pestivirus,* family *Flaviviridae*. There are two species of BVDV (BVDV 1 and BVDV 2) that can be further classified into subgenotypes (BVDV 1a – 1u and BVDV 2a – 2d) (4,11). In cattle herds, BVDV is one of the most widespread pathogens (6), associated with respiratory and reproductive problems (11). Immunosuppression can occur in the acute period of BVDV infection resulting in increased susceptibility to secondary infections (2).

BVDV has also been found in other animal species, such as sheep, goats, bison, and pigs (domestic swine and wild boars) (6,8). Considering the presence of BVDV in pigs and captive boars in Brazil (6,10) and the current situation of increasing wild boar invasion in South America, including Brazil, this study aims to investigate the presence of BVDV in free-living boars.

Materials and Methods

Lung tissue samples from 14 free-living wild boars were collected between the years of 2018 to 2019 in the Northern region of Paraná State, Brazil. Wild boars were captured during management by exotic wildlife controller agents properly authorized and registered in the Federal Technical Register of Potentially Pollutive Activity (CTF/APP-IBAMA). This research was supported by the Ethics Committee on the Use of Animals (CEUA/UEL – 22831,2017,40).

For the detection of BVDV RNA, the nucleic acid of the pulmonary tissue samples were extracted using the combination of phenol/chloroform/isoamyl alcohol (25:24:1) and silica/guanidinium isothiocyanate methods (1) and submitted to RT-PCR using primers designed to amplify a partial fragment of the 5´UTR region of the BVDV genome with 288 bp length (10). The amplified products were analyzed in 2% agarose gel stained with bromide of ethidium and visualized under ultraviolet light. The nucleotide sequences of the amplicons were determined to the classification of BVDV in species and subgenotypes.

Results

Of the total lung samples analyzed by RT-PCR in 3 (21.4%) were amplified RNA of BVDV. In sequence analysis, two amplicons showed similarities with BVDV-1d and one with BVDV-1a.

Conclusions and Discussion

This is the first report of the BVDV detection in free-living wild boars in Brazil and the first report of BVDV-1a subgenotype in wild boars in the world. The subgenotypes (BVDV-1a and 1d) found in the present tudy have been described as predominant in Brazilian cattle herds (3), this demonstrates that there was the contact of wild boars with bovine mainly with extensive beef cattle herds.

Considering the free movement of wild boars in the Brazilian territory and the possibility of contact with different cattle herds within the living area of the invasive species, there is a risk of spreading the BVDV for cattle or from cattle to wild boars.

The BVDV infection in pigs and wild boars is asymptomatic and, therefore, the virus can remain in wild boar populations that may represent a risk factor for cattle herds (5). This important epidemiological aspect of the BVDV infection in the wild boar population may hinder the control and prophylaxis of BVDV in cattle, for example, by vaccination (7).

The results of this report indicate that in the studied region, wild boar can be a source of infection of different BVDV subgenotypes for the neighboring cattle herds. However, studies including other important regions of Brazilian livestock should be carried out to understand the role of free-living boar in the dissemination of this virus for the cattle herds.

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Serosurvey for Pseudorabies (Aujeszky's Disease) in Free-Range Wild Boars of Brazil

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Introduction

Pseudorabies or Aujeszky's disease, caused by the pseudorabies virus (PRV), has been considered a notifiable and contagious livestock disease[1]. This disease is often associated to high mortality in piglets and abortion[1]. Domestic pigs (*Sus scrofa domesticus*) and wild boars (*Sus scrofa*), members of family Suidae, have been recognized as natural hosts of PRV[2]. In Brazil, wild boars have been classified as exotic invasive species, originated from Eurasian wild boars and their hybrids, and nationwide hunting is officially permitted as a strategy for population control and eradication[3]. Although PRV infection is reportedly established in wild boars elsewhere, no study has been conducted in free-ranging wild boars of Brazil. Our aim was to determine the presence of PRV antibodies in wild boars from different areas of two Brazilian biomes.

Materials and Methods

The study was conducted in preserved and degraded areas in the Atlantic Forest biome of southern Brazil, including the Vila Velha State Park (25°12'34"S, 50°03'37"W), and in degraded areas in the Cerrado biome of central-western Brazil, at the Aporé municipality (18°57′54″S, 51°55′33″W).

Free-ranging wild boars from degraded areas were sampled following hunting by firearm, under the Brazilian hunting laws, by legally registered hunters at the Brazilian Institute of the Environment and Renewable Natural Resources. Freeranging wild boars from a natural area in the Vila Velha State Park were baited, photo-monitored, trapped, and shot, following authorization by the Environmental Institute of Paraná (number 30.17).

Blood samples were collected from 94 animals by intracardiac puncture immediately after death from October 2016 to May 2018. The presence of antibodies against PRV was performed at the Biological Institute, a National Livestock Reference Laboratory (São Paulo, Brazil) with wild boar serum samples were tested in duplicate by a commercially available enzyme-linked immunosorbent assay (IDEXX PRV/ADV gB Ab Test®, IDEXX Laboratories, Westbrook, Maine, USA) and results interpreted as positive or negative. Enzyme-linked immunosorbent assay sensitivity has been considered the international gold standard serological method for PRV diagnosis and notification by the World Organization for Animal Health (World Organization for Animal Health 2018).

Results

Antibodies were detected in 0.03% (1/36) adult free-ranging wild boars from Cerrado biome of central-western Brazil. None of the 48 wild boars from Atlantic Forest biome degraded areas of southern Brazil were seropositive to PRV antibodies.

Conclusions and Discussion

As PRV transmission to secondary hosts may occur by secretions and excretions of infected suids[5], the seropositive wild boar in this study suggests the low PRV circulation in degraded areas of central-western Brazil, a large area of susceptible livestock, domestic animal and wildlife coexist. Despite serological PRV detection herein, further studies should be performed to molecularly characterize and isolate wild swine PrV, as well as to fully establish the risk of PRV spillover infection to domestic pigs.

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Genetic diversity of influenza A virus in pigs at weaning in Midwestern United States swine farms

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Introduction

Piglets play an important role at maintaining influenza A virus (IAV) infections in breeding herds and disseminating them to other farms at weaning. However, the role they play at weaning to support and promote genetic diversity of IAV is not fully understood. Therefore, we evaluated the magnitude of genetic diversity of IAV in pigs at weaning in farms located in the Midwestern US.

Methods

Nasal swabs (n=9,090) collected from piglets in breeding farms (n=52) over a six month period across seasons were investigated for the presence of IAV. Nasal swabs (n=391) from 23 IAV positive farms were whole genome sequenced, and genetic variability of IAV was quantified within and between farms by four different approaches. These approaches included: 1) analysis of the whole genomes derived from 126 nasal swabs at the genotype level based on the gene constellation of the complete sequences of all the 8 segments, 2) evaluation of the level of significance of genetic diversity operating between the farms based on all complete sequences available per segment using AMOVA (Analysis of Molecular Variance), 3) analysis of the presence of distinct genetic clades or clusters for all segments by inferring ML trees, and 4) investigation of the level of variability between farms for all complete sequences obtained using Shannon-Weiner diversity index.

Results

Multiple lineages of HA (n=7) and NA (n=3) were identified in 96% (22/23) and 61% (237/391) of the investigated farms and individual piglets, respectively. Co-circulation of multiple types of functional HA and NA were identified in most (83%) farms. Whole IAV genomes were completed for 126 individual piglet samples and 25 distinct and 23 mixed genotypes were identified, highlighting significant genetic variability of IAV in piglets. Co-circulation of IAV in the farms and co-infection of individual piglets at weaning was observed at multiple time-points over the investigation period and appears to be common in the investigated farms. Statistically significant genetic variability was estimated within and between farms by AMOVA and varying levels of diversity between farms detected using the Shannon-Weiner Index.

Discussion

Results reported here demonstrate previously unreported levels of molecular complexity and genetic variability among IAV at the farm and piglet levels at weaning. Movement of such piglets infected at weaning may result in emergence of new strains and maintenance of endemic IAV infection in the US swine herds. Our discoveries highlight the need for developing and implementing novel, effective strategies to prevent or control the introduction and transmission of IAV within and between farms in the country.

Antigenic analysis: an essential tool to select and update influenza A virus vaccine strains

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Introduction

The vaccination is the main measure to control influenza A virus (IAV) in commercial swine farms[1], being imperative the selection of suitable vaccine strains and a continuous surveillance to evaluate their update[2]. Accumulation of mutations in the antigenic sites of the hemagglutinin (HA) can allow the virus to escape the immune control and emerge as a novel epidemic strain[3,4]. The objective of this study was to analyze IAVs from different H1 clusters by antigenic analysis and determine if the genetic analysis is able to predict the emergence of antigenic variants.

Materials and Methods

Thirty-eight H1 IAV strains and their HA sequences were obtained from 21 swine farms in Chile. Of these, 13 strains were genetically selected to produce antisera, which represented different farms, companies, and geographical regions. Hemagglutination inhibition (HI) assays were performed by testing the antisera against the 38 IAV strains, following a standard protocol[5]. The HI results were used to construct a three-dimensional (3D) antigenic map (www.antigenic-cartography.org/). A genetic analysis was performed based on HA1 domain amino acid sequences to determine the genetic basis for antigenic differences. A 3D genetic map was made with Multidimensional Scaling method from a dissimilarity matrix based on HA1 amino acid sequences, using the XLSTAT software (version 2018.1). The antigenic and genetic clusters were defined by Ward's method based on the Euclidean distances among strains. Finally, HA 3D structures were generated to visualize key amino acid substitutions, using PyMOL Molecular Graphics System (version 2.1.1).

Results and Discussion

Three antigenic clusters were identified, named Chilean H1 A (ChH1A), Chilean H1 B (ChH1B), and pandemic H1N1 2009-like (A(H1N1)pdm09-like). Strains with the broadest cross-reactivity within each antigenic cluster were detected, which could be selected as potential vaccine candidates. Notably, some ChH1A and A(H1N1)pdm09-like strains were antigenically distant from their respective antigenic clusters (antigenic variants). The genetic analysis was not able to predict the emergence of antigenic variants from the cluster A(H1N1)pdm09-like. Amino acid substitutions in the main antigenic sites (E153G in Sa, Q193H in Sb, D168N in Ca1, P137S in Ca2, and F71L in Cb) were detected in ChH1A antigenic variants, whereas only a single substitution in the antigenic site Sa (G155E) was detected in A(H1N1)pdm09-like antigenic variants. The presence of various substitutions in the ChH1A antigenic variants could explain the genetic divergence of these viruses in the genetic analysis, unlike A(H1N1)pdm09-like antigenic variants, whose antigenic differences were not appreciated in that analysis.

Conclusions

Antigenic variants have mutations in antigenic sites that may interfere with neutralizing antibodies. The A(H1N1)pdm09-like antigenic variants were not able to be predicted by genetic analysis, emphasizing the need to carry out antigenic analysis to detect antigenic variants. Furthermore, the antigenic analysis permits to detect the strains with the broadest cross-reactivity, which can be used as vaccine strains.

Acknowledgments

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Persistent infection and protection against Senecavirus A in pigs previously infected with a homologous and a heterologous isolate

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Introduction

The emergence of a vesicular disease in pigs in 2014/2015, caused by Senecavirus A (SVA), has been responsible for great concern due to its high similarity with reportable diseases, such as foot-and-mouth disease. Despite the recent onset of SVA outbreaks in pig farms from different countries, the virus has been shown to be silently circulating in U.S. herds since at least 1988[1,2]. Preliminary data demonstrated the presence of virus in the tonsils of naturally-infected pigs approximately 90 days after showing clinical disease, which suggests a potential for establishing persistent infections and an asymptomatic carrier state. In addition, there was no evidence whether animals previously exposed to a SVA strain are protected against homologous and heterologous isolates.

Materials and Methods

Twenty-eight three weeks old crossbred gilts were allocated in three groups: group HC, inoculated with a historical followed by a contemporary isolate (n=12); group CC, inoculated with a contemporary isolate in the two different time-points (n=12); and group N, inoculated with RPMI medium (n=4). Historical and contemporary isolates were obtained in 1999 and 2017 respectively. One animal from group CC died from an SVA-unrelated cause and was necropsied at 39 dpi. Two pigs from group N, 3 from group CC and 4 from HC were euthanized and necropsied at 49 dpi. The remaining pigs from groups HC and CC were re-challenged 50 days after the first inoculation. Oral swabs, fecal swabs and sera were collected for RT-qPCR, and sera was also submitted to indirect immunofluorescence (IFA) and serum neutralization (SN) assays. Shedding was determined when either oral or fecal swabs were positive by PCR. The time-points for collections were 1, 3, 7, 10, 14, 21, 28, 35, 42, 48, 52, 55, 57, 64 and 71 days post first inoculation (dpi). At day 71, the remaining pigs from all groups were necropsied. Tonsils of the soft palate, submandibular and retropharyngeal lymph nodes from all pigs were submitted for PCR.

Results

All animals from groups HC and CC were shedding at day 3 dpi. Shedding started decreasing at days 10 and 14dpi, with the last detections on days 28 and 35dpi, for groups HC and CC, respectively. Viremia started at 3dpi in both infected groups (12/12 HC and 11/12 CC) and persists until days 28 for group HC and 21for group CC (Figure 1). Serological IgG response was first detected at 10 and 14dpi in HC and CC groups, respectively (Figure 2).

Two days after the second inoculation 3/8 oral and 8/8 fecal swabs from both groups were positive by PCR, but there were no positive swabs in the following time-points. Viremia was observed in one pig from each of the HC and CC groups at 7 and 5 days after re-challenging, respectively. All pigs from groups HC and CC had at least one of the three collected tissues positive by PCR after necropsy. Serum neutralization assays are in progress and will be completed before the IPVS meeting.

Conclusions and Discussion

Viral shedding after the first inoculation persisted up to 28 and 35 days in HC and CC groups, respectively, while viremia was consistently detected for 28 days from HC and 21 days from CC group. However, positive PCR was only detected at two days after the second inoculation, with fewer animals (3/8 HC, 3/8 CC) being positive in the oral swabs, indicating that the higher positivity rate in the fecal swabs at this moment may be due to detection of the inoculum. These findings suggest that both groups re-challenged with homologous and heterologous strains showed protective immunity, characterized by limited shedding. PCR results from collected tissues after necropsy suggest potential persistent infection in pigs with SVA. The role of persistently infected animals on the transmission of SVA infection should be further evaluated.

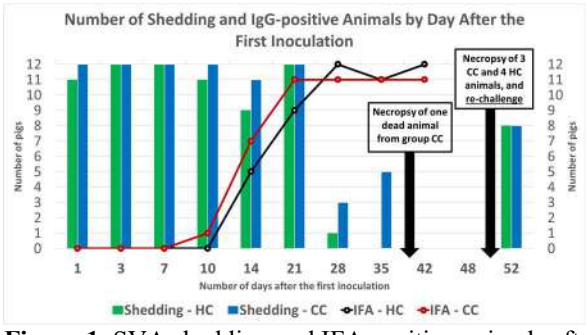

Figure 1. SVA shedding and IFA positive animals after the first inoculation.

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Prevalence of PCV2 genotypes in Brazilian herd samples during 2018 – 2020

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Introduction

Porcine circovirus type 2 (PCV2) is a single strain DNA (ssDNA) with higher substitution/evolution rate (1), which could explain the existence of six different genotypes (1). These genotypes has antigenic differences which could lead to immunological scapes (1). The aim of this study were to evaluate the actual distribution of the different PCV2 genotypes in Brazilian herd samples during the last two (2) years (2018 – 2020).

Materials and Methods

59 oral fluid samples were collected from the main producing regions of the country, DNA was extracted. ORF2 region from PCV2 genome sequences were translated into aa using Expasy program (3). ORF2 sequence from PCV2a, PCV2b and PCV2d were obtained from (4). Those sequences were aligned by (5). Molecular modeling of sequence representative each genotype of PCV2 ORF2 were performed using (6) and the capsid protein monomer model of the subtypes of PCV2 were built using as template the structure (PDB ID: 3R0R:A) (7).

Results

From the 59 oral fluid samples 40 (Forty) samples were from 2018 and 19 (nineteen) from 2019-2020. Samples from 2018 had fifty percent (50 %) from PCV2b and PCV2d. Therefore samples from 2019-2020 were 100% (19/19) from PCV2d isolates.

Conclusions and Discussion

Beside the fact that these results are a preliminar results, those data may show some interesting points. PCV2a are not being seeing in the field since 2016 (9) which could explain the theory about PCV2 genotype evolution (0/59), emerging novel genotypes. These results corroborate with (8) and (10) that characterize quite similar the process of genotype evolution. Another interesting point is about PCV2d that increase prevalence in accordance with other researchers (8), being the only predominant genotype in 2020 in Brazilian data. These results demonstrate that PCV2 are a dinamic virus that has a very fast evoluion rate. This could be explained when compared data from recent study (9) showing an expressive evolution rate, since in 2005 the prevalente genotype were PCV2a and PCV2b.

This study concludes that the PCV2 evolutionary rate allows a faster evolution rate, and it's still very important keep colecting samples at the field and monitoring these data frequently, in order to check the evaluation os this new genotypes.

Table 1. Distribution of prevalence and frequency from different PCV2 genotypes within 2019 to 2020.

Ano das amostras	N total	PCV2a		PCV2b		PCV2d	
		n	%	n	%	n	%
2018	40	0	0,0 (0/40)	20	50 (20/40)	20	50 (20/40)
2019-2020	19	0	0,0 (0/19)	0	0,0 (0/19)	19	100% (19/19)

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Productive improvement on PRRSv co-infected swine farms in Thailand vaccinated with UNISTRAIN® PRRS ID

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a global viral swine disease that causes economic losses roughly estimated at 32.9 million US$ annually. The use of modified live vaccines (MLV) to stabilize the breeding herd is a widespread practice.

UNISTRAIN® PRRS, by either the intradermal (ID) or intramuscular (IM) route of administration, can induce both humoral and cell-mediated immune responses against a HP-PRRSv infection and even in a co-challenge situation with type 1 PRRSV (1).

The aim of this study was to evaluate the field efficacy and safety of UNISTRAIN® PRRS MLV vaccine on a PRRSv1 and PRRSv2 co-infected farm with a recent outbreak recorded.

Materials and Methods

The study was carried out on a 12,000-sow farrow-to-wean farm located in a high swine density area in the southern part of Thailand. The mortality rate in lactation and fattening units was 9-14% and 15-20%, respectively. PCR diagnostic assay (HIPRA Diagnos® Laboratory) confirmed PRRSv1 and PRRSv2 infection on the farm. The farm owners decided to start using UNISTRAIN® PRRS due to the PRRSv instability situation and the ongoing losses of grower-finisher pigs. Sows were intradermally vaccinated with the registered vaccine combination for PRRSv and Aujeszky´s disease (UNISTRAIN® PRRS + AUSKIPRA® GN) using a needle-free injector HIPRADERMIC®). Piglets were intradermally vaccinated at 7-days of age with UNISTRAIN® PRRS using HIPRADERMIC®. The injection site was examined by palpating for local reactions daily until 3 days post vaccination. The efficacy of UNISTRAIN® PRRS in controlling PRRSv1 and PRRSv2 coinfections was determined by a 1-year period comparison of the reproductive and productive parameters before and after the use of UNISTRAIN® PRRS was started. (SPSS statistical program, version 22.0).

Results

No adverse reactions were observed in sows and piglets after ID vaccination with UNISTRAIN® PRRS, apart from the expected papule, which returned to normal three days after vaccination (Fig 2). Pig losses (stillborn + dead piglets + euthanised piglets) and weaned piglet weights showed a significant improvement after starting to use UNISTRAIN® PRRS. The farrowing rate, the born alive piglets and the number of weaned piglets were slightly higher with UNISTRAIN® PRRS compared to the previous one-year period (Table 1).

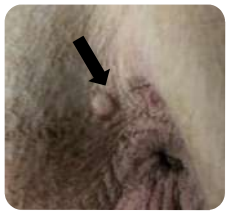

Figure 2. Observed papule in sows after intradermal vaccination with Hipradermic®

Table 1. Comparison of a 1-year period before and after the use of UNISTRAIN® PRRS.

Production parameter	2018	UNISTRAIN® PRRS (2019)	Diff
Farrowing rate (%)	85.8±3.3[a]	87.9±3.3[a]	+2.1
Born alive piglets/litter	10.1±0.3[a]	10.3±0.3[a]	+0.2
Weaned litters/sow/year	2.4±0.03[a]	2.5±0.1[a]	+0.1
Weaned piglets/sow/year	23.7±0.9[a]	24.7±1.6[a]	+1
Pig losses (%)	11.1±3.3[a]	9.2±3.3[b]	-1.9
Weaned piglet weight (at 25 days old) (kg)	6.8±0.3[a]	7.4±1.04[b]	+0.6

[a,b] different superscripts indicate statistically significant differences ($p \leq 0.05$)

Conclusions and discussion

In the same production system, UNISTRAIN® PRRS was shown to be a useful tool to control of PRRSv 1+2 field infections, increasing reproductive and productive parameters. It is well known that the ORF5 homology between the field isolates (PRRSv 1 and 2 in this case) and vaccine strain (PRRSv1 in this case) is not a predictor of the degree of protective immunity conferred and (2). This field study shows the cross-protection conferred by UNISTRAIN® PRRS to be a effective measure in a co-infection situation. Future studies should be performed to collect more production data on animals of all ages after the long-term use of UNISTRAIN® PRRS on this co-infected farm.

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Application of disinfectant on the udder skin of sows to decrease levels of influenza A virus contamination at weaning

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Introduction

Influenza A virus (IAV) causes respiratory disease and is endemic in swine herds. Piglets are considered a susceptible population and are responsible for maintaining influenza virus in breeding herds (1). Some management practices, including use of nurse sows, are used to ensure suitable milk intake of piglets to improve piglet viability. However, use of nurse sows has been implicated in the transmission of IAV among litters prior to weaning (2). Therefore, the aim of the present study was to evaluate whether using a disinfectant on the udder skin of nurse sows at weaning could decrease the level of IAV contamination in the udder skin of the sows.

Materials and Methods

This study was approved by the University of Minnesota Institutional Animal Care and Use Committee (Protocol No. 1705-34808A). Seventy-eight lactating sows were enrolled at weaning and randomly distributed in three groups (Table 1).

Table1. Distribution of sows on treatment groups

Group	n	Name	Intervention
1	25	Treatment 1	Disinfectant
2	23	Treatment 2	Water
3	25	Control	No intervention

In Group 1, a quaternary ammonia based disinfectant was sprayed on the surface of the udder skin of each sow covering the underline for approximately 20 seconds after piglets were weaned off. In Group 2, a similar procedure was followed using only water. Lastly, no intervention was applied to Group 3 which served as untreated control group. Udder skin wipes were collected from each sow before (1st sampling event) and 30 minutes (2nd sampling event) after each treatment. All samples were tested for IAV by RT-PCR targeting the matrix gene as previously described (3). Samples Ct≤37.5 were considered positive and samples with Ct<35 were cultured for virus isolation. The number of positive samples before and after each treatment were analyzed using McNemar test and differences in Ct values were evaluated using Wilcoxon signed rank test (alpha=0.05).

Results

Proportion of positive samples and Ct values are shown on Figure 1 and 2, respectively. The number of IAV positive samples on the udder skin decreased in Group 1 after the skin was treated with disinfectant (p=0.023). This reduction was not observed in Group 2 where water was used instead of disinfectant or Group 3 which was the untreated control. In Group 1, Ct values were higher on the second sampling compared to the first one (p=0.001) indicating that the application of the disinfectant was effective at reducing IAV on the udder skin. Four IAV were isolated, including one each from groups 1 and 3 on the first sampling event and one each from groups 2 and 3 on the 2nd sampling.

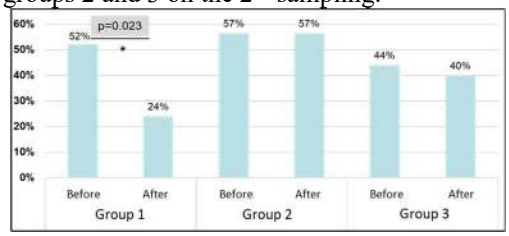

Figure 1. Prevalence of IAV on the udder skin of sows before and after each treatment.

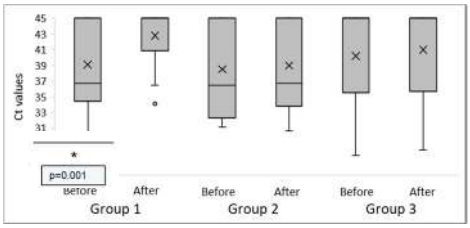

Figure 2. Ct value distribution of IAV on the udder skin of sows before and after each treatment.

Conclusions and Discussion

Our study confirms prior findings that indicate that sows at weaning can be a source of IAV infection. In addition our findings also indicate that topical disinfectants can help reduce the prevalence of sows with contaminated udder skin. To the knowledge of the authors, this is the first study that reports the use of a disinfection procedure to decrease the detection of IAV on the udder skin of sows at weaning. Additional studies are required to validate protocols with different disinfectants and to evaluate whether such interventions will decrease transmission of IAV to piglets upon adoption.

Acknowledgments

University of Minnesota Swine Disease Eradication Center and Pipestone Veterinary Services.

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WELFARE

A longitudinal study of umbilical outpouching in Danish pigs

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Introduction

Umbilical outpouchings (UO) are common in pigs worldwide. The main pathological manifestations causing UO are: 1) herniation, 2) enterocystoma, 3) peritoneal proliferations, 4) abscesses, and 5) fibroses. These manifestations appear either alone or in combination.

Pigs with UO often do not reach the abattoir as they die due to complications or are euthanized due to welfare concerns.

The objectives of the study were to estimate the distribution of hernia umbilicalis and other pathological manifestations causing UO, and to investigate if UO in some pigs disappear clinically before slaughter. Secondly, the association between pathological manifestations of UO and the cause of death was analyzed.

Materials and Methods

A longitudinal study following pigs from birth to slaughter was carried out in two commercial Danish farrow-to-finisher herds with a history of a high prevalence of pigs with UO.

A total of 3031 piglets from 161 litters were included in the study. Piglets were inspected at farrowing, in the second week after birth and every month afterwards for the development of UO (height > 0.5 cm). Animals with OU were clinically examined monthly until slaughter or death. Measurements such as size, colour, shape and presence of lesions together with reducibility of the content were obtained.

Due to animal welfare, pigs were euthanized if: 1) lesions were identified on the UO, 2) the size of the UO was affecting gait and movement, or 3) general conditions were affected. When pigs with UO died spontaneously, were euthanized or slaughtered, necropsies were performed to clarify the cause of the UO.

In order to investigate the association between umbilical diagnoses and the cause of death a Chi square test was applied. (RStudio Team, version 1.0.153).

Results

In total, 255 (8.4%) pigs developed UO; of which 37 (14.5%) spontaneously disappeared before slaughter. 137 (53.7 %) of the pigs died or were euthanized before slaughter, and 103 (40.4%) were slaughtered. 15 (5.9%) pigs were missing (lost earmark, dead, moved etc.).

During the study period, 208 (81.6%) pigs were examined post-mortem, and 34 (16.3%) pigs had no pathological manifestations in the umbilical region. The main pathological cause of UO were hernia umbilicalis (54.3%). In 72 pigs with hernia, it was the only diagnosis and in 41 pigs, the hernia appeared in combination with other lesions. In total, 29.3 % of the pigs were diagnosed with enterocystoma, proliferations or an abscess/fibrosis.

Table 1. The distribution of 174 pigs with either hernia or other pathological manifestations of UO and the cause of death.

DIAGNOSIS	**CAUSE OF DEATH** (**n** (%)) Dead/ Euthanized	Slaughter	Total
Hernia	97 (85.8)	16 (14.2)	113(100)
Other UO manifestations	38 (62.3)	23 (37.7)	61 (100)
Total	135	39	174

The odds of dying before slaughter were significantly higher among pigs with an umbilical hernia (OR= 3.67, P= < 0.001) compared to pigs with other pathological manifestations causing UO.

Conclusions and Discussion

Umbilical herniation was the main pathological manifestation causing UO in this study, and in some pigs, UO disappeared clinically before slaughter.

Pigs with umbilical hernia were at a higher risk of dying before slaughter compared to other pathological manifestations causing UO.

Identifying the different pathological manifestations in pigs with UO and their ability to reach slaughter is fundamental for optimizing prevention and treatment of pigs with UO.

Behavior and reproductive performance of primiparous and multiparous sows using different feeding systems in gestation group housing

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Introduction

Despite the positive acceptance of gestation group housing in good production practices as an animal welfare strong ally, there are several doubts regarding its reproduction impact (1). The purpose of this study was the **evaluation** of the behavior at feeding time and the reproductive performance of pregnant sows in group housing equipped with different feeding systems.

Materials and Methods

Post-weaning primiparous and multiparous sows had their estrus identified and were artificially inseminated. After the artificial insemination protocol, the sows were housed in groups of 9 animals during the gestation period, in stalls with 2,25 m²/sow, where the groups were identified as: **P0CSY:** sows in group housing using "Y" drops feeder; **P0CSMB:** sows in group housing using minibox feeder; **P1CSY**: post-weaning primiparous sows in group housing using "Y" drops feeder; **P1CSMB**: post-weaning primiparous sows in group housing using minibox feeder.
At the "minibox" system (MB), the stalls had partitions (48x50 cm, width/depth), with nine partitions per stall, one for each sow, so they could eat alone, decreasing any fighting for food. At the "Y" drops feeding system, the food dropped at the floor without the segregation of spaces, so there could be fighting. Ten observations were made for each group. The stalls were identified as experimental units. At the gestation period, the sows' behavior parameters were evaluated (exploration, vocalization, agonistic, social, positive and active behavior and others) at feeding (2). Two evaluations per stall were made with a two minutes break (at the beginning of feeding and two minutes after its start). At farrowing, several indexes were registered: farrowing rate (FR), total piglets born (TB), piglets born alive (BA), stillbirth (SB) and mummified fetuses (MF). The number of each sow behavior observed was converted in percentages for the ANOVA evaluation, testing the system's results (MB vs Y) of the evaluation and the interaction time. The data were analyzed using the Statistical Analysis System (SAS®) software, 9.4 version, for both systems (MB vs "Y). The GLIMMIX procedure was applied considering the negative binomial analysis for the farrowing rate. TB and BA were compared using the Tukey-Kramer test. SB and MF were evaluated with the NPAR1WAY procedure. The differences were considered significant using the probability level of 95% (p<0,05). This study was approved by the Ethic Committee for Animal Use of the IFC Araquari Campus (Protocol 265/2018).

Results

No differences were found between the feeding systems for multiparous (Table 1) and primiparous (Table 2) sows in group housing. The percentage of sows with active behavior (eating or drinking) was smaller (p<0,05) at the "Y" system in comparison to the MB system. For the agonistic behavior (aggressive behavior among sows, such as pushing, biting and fighting), the results were worse for the primiparous sows using the "Y" system (p<0,05). There were no differences among the other behavior parameters (p>0,05) for the primiparous and multiparous sows.

Conclusion and Discussion

The "Y" feeding system, without partitions, had no negative impact at the reproductive performance of the primiparous sows, although there was an increase in agonistic behaviors, and fewer active sows at feeding.

Table 1. Multiparous sows performance at the different feeding systems (n=10, stalls with 9 sows; mean ± Standard Error Mean).

System	FR	TB	BA	SB	MF
P0CS	89,02	14,00	12,73	6,03±	2,61±
MB	±0,03	±0,54	±0,45	1,43	0,58
P0CSY	88,51	14,14	13,19	4,57±	2,12±
	±0,03	±0,54	±0,45	1,43	0,58
Pr > F	0,9174	0,8293	0,4959	0,4538	0,5332

Table 2. Primiparous sows' performance at the different feeding systems (n=10, stalls with 9 sows; mean ± Standard Error Mean).

System	FR	TB	BA	SB	MF
P1CS	95,39	13,62	12,16	5,20±	5,48±
MB	±0,02	±0,40	±0,39	1,03	1,23
P1CSY	93,24	13,59	12,55	4,25±	3,33±
	±0,03	±0,40	±0,39	1,03	1,23
Pr > F	0,5578	0,9403	0,4509	0,5310	0,2401

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Effect of local anesthesia and/or analgesia on pain response induced by piglets castration

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Introduction

Consumer pressure is increasing on agribusinesses to respect animals' sentience, driven not only by technological quality of animal products, but also by ethical appeal and animal welfare (2,4). The aim of the study was to evaluate the behavior of piglets submitted to castration under anesthetic and/or analgesic protocols.

Materials and Methods

This study was approved by the Ethics and Animal Welfare Committee of the State University of Northern Paraná under certificate number 06/2018. 92 piglets were handled and distributed in four treatments: T1 – Control (without medication), T2 – Lidocaine 2% (0,5 mL intratesticular/testis), T3 – Meloxicam 0,2% (0,4 mL intramuscular) and T4 – Lidocaine and Meloxicam (volumes previously described) with 23 repetition per treatment. The animals were spayed at 7 days of age, 15 minutes after applying the medication. During castration, the presence or absence of movements of the anterior and posterior limbs, emission of urine or feces and the occurrence of vocalization through crying or silence were observed. One hour after castration, the presence or absence of prostration (immobility), tremors, tail movements, isolation, sleeping or nursing was observed. Blood collection for cortisol analysis was performed after a behavioral analysis of 1 hour after castration. The blood samples were centrifuged to collect 1,5 mL of serum, which was stored in a freezer at 20º C. To check the differences between treatments, analysis of variance was performed, considering the level of 5% probability.

Results

Behavioral assessment during castration, showed that there was less movement of the anterior and posterior limbs of the animals treated with lidocaine (17/23) compared to the control treatment (23/23). Vocalization (crying) was decreased in animals treated with Lidocaine (20/23) compared to other treatments (23/23). One hour after castration, there were no differences in prostration, tremors, tail movements, isolation, sleeping, nursing and lying down. Blood cortisol levels, collected 1 hour after castration, showed no differences in the mean values in the different treatments. (Table 1)

Table 1. Average cortisol values (ng/mL) for different types of treatment

Treatment	Cortisol (ng/mL)
Lidocaine	85,54[a]
Control	93,45[a]
Meloxicam	100,55[a]
Lidocaine + Meloxicam	103,18[a]

Discussion and Conclusion

According to (3), the pain in surgical procedures can be reduced with the application of anesthesia, however, it is not effective to eliminate discomfort and stress generated by castration, which corroborates the result found in this study, Our results showed that, despite decreased movements of the anterior and posterior limbs in animals treated with lidocaine alone, no changes in blood cortisol levels 1 hour after castration was observed. This is likely due to the stress generated by all the handling during castration. According to (1), piglets suffer from pain for several hours after surgical castration and emphasizes the need to develop anesthetic and analgesic protocols that reduce pain and associated behaviors. It can be concluded that the use of anesthetic decreased the movements of the anterior and posterior limbs and vocalization during castration, but did not decrease the level of blood cortisol.

Acknowledgments

Thanks to Fundação Araucária for the scholarship granted to carry out this project and the State University of Northern Paraná.

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Severe vulva biting in gestating sows; a case report

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Introduction

Vulva biting is a welfare impeding behaviour, especially seen in group housed sows fed with electronic sow feeders (ESF's). Known other risk factors are: restricted opportunities for foraging, nearby presence of a boar, use of nipple drinkers (1,2) low satiety (3), tail biting in rearing gilts (1) and high stocking density (2).
At a 1200 head SPF sow farm in the Netherlands, where 800 sows were housed in one dynamic group with 14 ESF's, periods with severe vulva biting were observed.
This study aims to describe in this farm the incidence of vulva biting and to study associations with parity and production stage as well as to describe the diagnostic process and its results to detect potential causes and recommend improvements in this farm.

Materials and Methods

First, a random sample of 255 sows in the farrowing unit and 55 gilts in the gilt rearing area and gestating gilt pen were examined in detail for acute or chronic lesions due to vulva biting. Descriptive data analysis was performed.
Next, continuous 2x24h video capture of the area near the entrance of 5 feeding stations was analysed in order to determine when, where and which sow(s) performed vulva-biting and what induced this behaviour. Periods in which less than four animals were waiting to enter the ESF were excluded from the analysis. Before detailed video analysis, labels specifying behaviour were defined. For a two-day period, every hour during seven minutes, vulva biting, and related behaviours and clinical signs, were quantified.
Finally, the farm was inspected for the risk factors known from literature.

Results

Chronic vulva lesions were observed in 158/230 (69%) sows of parity >1, but in 0/25 gilts in the farrowing room and in 0/55 gilts in the area for gestating gilts. However, this data did not exclude that potentially gilts were the offenders.

Video analyses revealed that biting was performed in the area near the entrance as well as in the feeding station. Vulva biting was only seen just after a new feeding day was started, particularly in the first nine hours. The analysis showed that overcrowding, residual feed (due to no water supply and or feed disposal rate) in the feeding station and subtle malfunction of the entrance gate were evident risk factors that contribute to vulva biting.
As we only observed biting after start of a new feeding day and that gilts were not affected it was concluded that the older and thus dominant sows are vulva biting. We neither observed gilts vulva biting.
Farm risk factor analysis revealed presence of all risk known risk factors as well.

We recommended to :

- Aim to detect the biting sows, usually the older dominant ones
- Stop overcrowding by removing older dominant sows;
- Repair entrance gate of the feeding stations
- Increase dietary fiber to adequate levels

Conclusions and Discussion

In addition to descriptive epidemiological analyses, video analyses showed pivotal to diagnose the origin of the problem.

Acknowledgments

The herd veterinarian and the farmer are greatly acknowledged for their assistance, advise and access to the farm and farm data.

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Investigation of the usage of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) in sows on 690 pig farms in Switzerland

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Introduction

Along with vaccinations, antimicrobials and antiparasitic drugs, NSAIDs are among the most frequently used preparations in sows in modern pig production. Main indications are the alleviation of fever and pain caused by infectious diseases or injuries; i.g. diseases of the locomotory system or the Postpartum Dysgalactia Syndrom (1,2,3). In Switzerland, on farms participating at the Suissano and Safety+ Health Program, all treatments with NSAIDs are recorded (4). The aim of the present study was to analyze the use of NSAIDs in gestating and lactating sows with regard to treatment frequency and main indications.

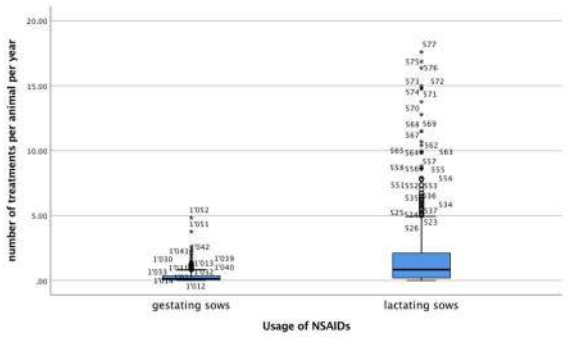
Materials and Methods

In 690 study farms, housing 14,000 lactating and 43,000 gestating sows, all treatments with NSAIDs between September 2018 and August 2019 were recorded using electronic treatment journals. For every study farm, the number of treatments per animal in this period was calculated and the main indications for treatments with NSAIDs were determined. Each administration of an NSAID was counted as one treatment.

Results

The number of treatments was 32211 in lactating sows and 12572 in gestating sows. Lactating sows were significant more frequently treated with NSAIDs than gestating sows ($p < 0.001$), with a median of the number of treatments per sow per year of 0.84 in lactating sows (maximum 17.6) and 0.13 in gestating sows (maximum 4.8). The main indication for NSAID treatments in gestating sows was lameness with a proportion of 87% of all treatments. In lactating sows, the main indications were related to the Postpartum Dysgalactia Syndrome (74%) and lameness (10%).

Table 1. Distribution of usage of NSAIDs (number of treatments) in lactating and gestating sows on the study farms.



Conclusions and Discussion

Treatments of sows with NSAIDs occurred regularly in the study farms and with varying frequency. For the future, it should be discussed whether the frequency of treatments with NSAIDs may be suitable for the use as an indicator for systems assessing animal health.

Acknowledgements

SUISAG, Allmend, CH-6204 Sempach

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Effect of intrascrotal administration of various local anesthetics for pain elimination during piglet castration

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Introduction

Worldwide most male piglets are castrated without pain relief during the first week of life to prevent the expression of boar odor (Frederiksen et al., 2011). Castration without pain elimination has been strongly criticized, especially in Western European countries with high animal welfare standards. In Norway since 2002, piglets have to be castrated under local anesthesia, and in Switzerland since 2010, under general anesthesia (isoflurane or ketamine/stresnil). In many European countries, painkillers are applied for postoperative pain control during castration. Alternatives to surgical piglet castration, such as entire boar fattening or vaccination against boar odor, have not yet been established in Europe. The intratesticular injection of procaine or lidocaine leads to additional pain, without significantly reducing castration pain (Zankl et al., 2007). Intrascrotal application of newer and more potent local anesthetics, which are frequently used in pediatrics, have not been comparatively investigated in pigs.

Materials and Methods

Our study was carried out in three farms according to the ethical guidelines of the University of Zurich and the Swiss Animal Welfare Act (experiment number ZH 090/18). All piglets were 2 - 5 days old and belonged to the breeds Yorkshire x Yorkshire or Yorkshire x Landrace, Yorkshire x Duroc or Yorkshire x Pietrain. Both the application of the local anesthetics and the castration, as well as the evaluation of pain expression, were carried out double-blinded. For this study, 492 male piglets were randomly divided into 10 groups of 50 resp. 42 piglets (Group A): Ropivacaine (3 mg/kg); B): Lidocaine (10 mg/kg) + Ropivacaine (3 mg/kg); C): phys. saline solution; D): Lidocaine 10 mg); E): Procain (6 mg/kg); F): Lido + Ropi + Epinephrine 1:100`000), castration after 45 minutes; G): Lido + Ropi + Epi, castration after 30 minutes; H): Lido + Ropi + Dexmetedomidine (5 mcg/kg); I: Lido + Ropi + Epi application dorsal of the testicles; J: Lido + Ropi + Epi application ventral of the testicles). Using an automatic syringe, 1.5 ml of the diluted local anesthetic was applied per side into the intrascrotal space between the vaginal process and the external spermatic fascia. The piglets were then placed on straw under a heat lamp and castrated after 45 and 30 minutes respectively. The defensive behavior (number and strength of limb movements as well as vocalizations) was evaluated independently by three veterinarians, according to a predetermined scoring system based on movie recordings during application of the local anesthetics as well as during the castration procedure.

Results

In terms of pain behavior, there was a broad consensus between the three independent vets (W=0.709). The pain score for Lido + Ropi and Lido + Ropi + Dexe were significantly lower ($p<0.014$) compared to all other groups. Surprisingly, the pain score for Lido + Ropi + Epi (ventral and dorsal application) was significantly higher $p<0.03$) than those of other groups. In all anesthetic groups, skin incision was the least painful step of the castration procedure with the majority of piglets showing little or no reaction. Pulling and cutting of the spermatic cord led to the highest pain scores in all groups.

Conclusions and Discussion

Since piglets were not sedated during either injection of the local anesthetic or castration, many piglets reacted independently of the intervention by screaming and defensive movements as soon as they were touched or fixed. Without sedation, it is almost impossible to fix piglets without defensive movements or the administration of local anesthetics. Piglets that already showed strong defensive movements during fixation tended to show higher, intervention-related pain scores in all sub-steps of castration (incision of the scrotal skin, protrusion of the testicles, cutting of the spermatic cord). Hence, piglet handling and a quiet castration site, are of great importance.
In our field study, it could be shown that all local anesthetics, administered intrascrotally, were able to provide pain relief but were unable to eliminate the pain reaction completely.

Acknowledgments

Many thanks to the Bavarian Animal Health Service for financing the field study and for the excellent cooperation.

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Influence of reproductive status on hematological parameters in multiparous sows of intensive production between two consecutive weaning in Chile

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Departamento de Patología y Med. Preventiva, Fac. Cs Veterinarias, Universidad de Concepción.

Introduction

Hematology is one of the tools that can provide valuable information regarding health of animals. However, in swine production it is rarely used due to the cost and lack of blood refence intervals (RI) (). Previous hematological studies of sows in different reproductive status are not common and have been carried out with small groups of animals, with emphasis at specific times during pregnancy or lactation (2, 3). There are some studies showing variations of red and white blood cells (WBC) between lactating and non-lactating sows (3). In addition to variations between lactating and pregnant sows (4). The absence of specific information between the different reproductive and productive stages of the sow, suggests further research towards an appropriate clinical diagnosis. Therefore, he aim of this study was to determine hematological parameters variations of multiparous sows in Chilean production between two consecutive weaning.

Materials and Methods

To do this, 307 healthy sows from two swine farms from Chile where chosen from to 8 farrowing. Tested six times, T at weaning, T2 at almost 24h before insemination, T3 at 30 days of gestation, T4 at 60 days of gestation, T5 at 90 days of gestation and T6 at second weaning.

The estimated blood parameters were count of red blood cells (RBC), hemoglobin concentration (HGB), hematocrit (Hct), total plasma protein concentration (TPP), platelets count (Pt), total leukocyte count (LEU) and its differential (eosinophils (EOS), lymphocytes (LIN), monocytes (MON) and neutrophils (NEU)) the results were analyzed using repeated measured ANOVA and Friedman's test.

Results

There was a significant increase ($p<0.05$) in red blood cells (RBC, HGB & Hct) by T3 and after a trend to decrease ($p<0.05$) to the second weaning (Fig.), similar to white blood cells, LEU, LIN & MON (Fig. 2). In case of NEU and TPP had higher values ($p<0.05$) at weaning (T & T6) (Fig. 2). At T5 WBC (Fig. 2) and Pt show a significant decrease ($p<0.05$)

Conclusions and Discussion

The variation of hematological parameters depending on reproductive status of the sows proves that the variations of red blood cells and white blood cells are concordant to previous studies (3, 4). These variations are because of the changing requirements of the growing fetus in gestation and hormonal variations in the sow to fulfill those requirements.

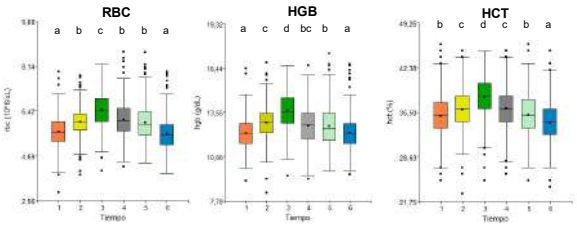

Figure . Hematological values of RBC, HGB and Hct of multiparous sows according to the time of sampling. Different letters by sampling times indicates statistically significant differences ($p<0.05$).

Difference In other studies in the total count of white blood cells associated with the increase of neutrophils induced by stress differs in this study

Complete blood count in Chile is like what was previously reported, despite environmental factors that may affect the hematological parameters. The difference in WBC count due to the increase of neutrophils may be due to avoiding sampling close to farrowing because of the increased stress that entails.

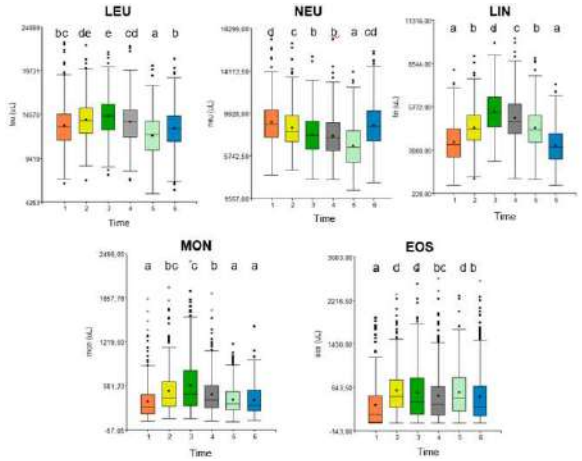

Figure 2. Behavior of WBC (LEU, NEU, LIN, MON & EOS) of multiparous sows according to the time of sampling. Red circled sampling times were not statistically significant.

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Influence of number of parturitions on hematological parameters in multiparous sows of intensive production between two consecutive weaning in Chile

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Introduction

Hematology is one of the tools that can provide valuable information regarding health of animals. However, in swine production it is rarely used due to the cost and lack of blood refence intervals (RI) (1). Previous hematological studies of sows in different reproductive status are not common and have been carried out with small groups of animals, with emphasis at specific times during pregnancy or lactation (2, 3). Variations between lactating and pregnant sows has been reported (4). There is an absence of specific information between the different parturition of the sow in Chile. The aim of this study was to determine the influence of farrowing number on the variations in hematological parameters of multiparous sows in Chilean production between two consecutive weaning.

Materials and Methods

In order to do this, 307 healthy multiparous sows with 1 to 8 farrowing from two swine farms from Chile where chosen

Sows were classified by parturition P1, P2, P3, P4 and ≥P5. They were tested six times, T1 at weaning, T2 at almost 24 hours before insemination, T3 at 30 days of gestation, T4 at 60 days of gestation, T5 at 90 days of gestation and T6 at second weaning.

The estimated blood parameters were count of red blood cells (RBC), hemoglobin concentration (HGB), hematocrit (Hct), total plasma protein concentration (TPP), platelets count (Pt), total leukocyte count (LEU) and its differential (eosinophils (EOS), lymphocytes (LIN), monocytes (MON) and neutrophils (NEU)) the results were analyzed using repeated measured ANOVA and Friedman's test.

Results

There was a decrease ($p<0.05$) in RBC, Hct (Fig. 1) and WBC parameters (LEU, NEU, and LIN) (Fig. 2) as the number of farrowing of the sow increased. The TPP had higher values ($p<0.05$) on sows with ≥5 parturitions, in all sampling events except T3. EOS, HGB, MON and Pt did not show statistically significant differences ($p>0.05$) between parities at different sampling times.

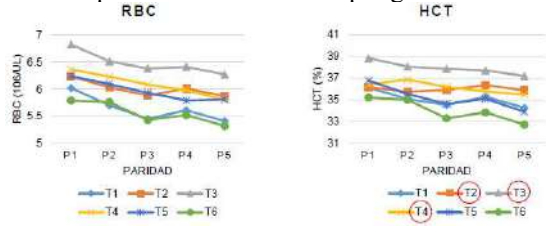

Figure 1. Hematological values of RBC and HCT of multiparous sows according to their number of farrowing. In times with a red circle, statistically significant differences were not found.

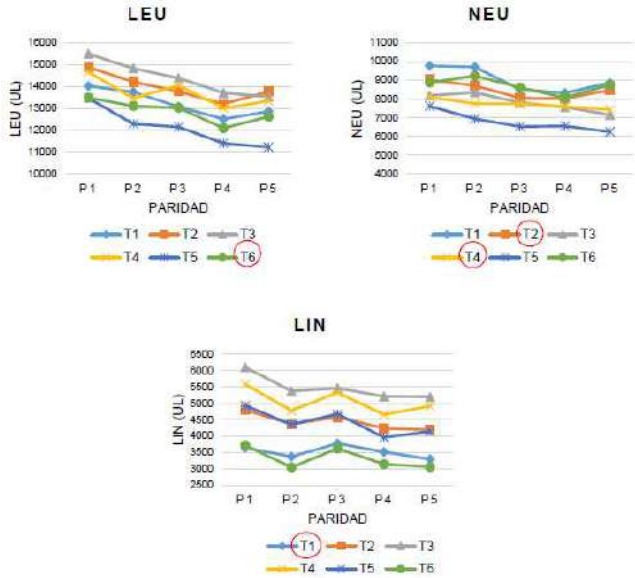

Figure 2. Hematological values of LEU, NEU, and LIN of multiparous sows according to their number of farrowing. Red circled sampling times were not statistically significant.

Conclusions and Discussion

The decrease of red blood cells and white blood cells is concordant with Ježek *et al*. (2018). In humans this phenomenon might be because of the reduction of hematopoietic stem cells, less hormonal stimulus and the decrease of space used by hematopoietic tissue in bone marrow (due to fat infiltration) (5). Given the similarity between humans and pigs, this may be the answer for this reduction.

The concentration of total proteins rises as farrowing increases, this have been described in other species as a physiological increase of globulins in plasma, this may lead this results in sows.

Further investigation is needed to understand the factors that are affecting these parameters in sows, and how to improve animal-welfare, by standardizing hematological parameters.

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Impact of transport distance and season of the year on transport-related mortality in finisher pigs

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Introduction

Transportation is the most stressful event for pigs prior to slaughter (1). Stress can result in pig fatigue, injury, poor meat quality and ultimately death (2). Mortality records during journeys and on arrival at slaughterhouses (death on arrival, DOA) as well as rates of carcass degradation are often the only data which give information about the possible welfare status of the animals during a journey and the severity of the problems encountered as death during handling and transport is usually preceded by a period of poor welfare (3).

The aim of this study was to assess mortality related to the commercial transport of finisher pigs for slaughter in the Czech Republic over a 6-year period, to determine the effect of transport distance and season (external air temperature) on pig mortality.

Materials and Methods

Data summarizing the numbers collected from all pig transport movements in the Czech Republic in the monitored period were gathered in the database of the Information Centre of the State Veterinary Administration. The percentages of transport deaths out of the total number of pigs transported within each category were calculated.

Data were analysed using the statistical package Unistat v. 6.5. (Unistat Ltd., London, England). Statistical comparisons between the frequencies of the categorical variables of interest were performed with the Chi-square test (with Yates correction) within the 2×2 Contingency table procedure. When the frequencies in the contingency table were lower than 5, a Fisher exact test was used instead of Chi-square test (4). A p value of 0.05 in tests was considered significant.

Results

Considering transport distance, the lowest mortality (0.049%) was found in pigs transported for distances below 50 km, the longer distances were associated with significantly ($p < 0.001$) increasing death losses with the highest losses (0.145%) recorded for distances exceeding 300 km.

Considering season, the highest mortality (0.075%) was associated with transport carried out in the winter months. Significantly ($p < 0.01$) lower death losses were associated with transport carried out in the summer months (0.070%). The lowest death losses were found in pigs transported for slaughter in the spring (0.066%) and autumn (0.066%) months; with no significant difference between these two seasons.

Transport carried out under the ambient temperatures –6 °C to –2.1 °C (the lowest temperatures observed in our study) and temperatures 18 °C to 21 °C (the highest temperatures observed in our study) were associated with the highest death losses of pigs. However, a significant difference ($p < 0.001$) was found only between the mortality rates related to transport carried out under the ambient temperatures –6 °C to –2.1 °C and mortality rates related to transport carried out under the ambient temperatures 2 °C to 17.9 °C. The mortality related to transport of finisher pigs carried out under a temperature range from –2 °C to 21 °C did not significantly differ.

Conclusions and Discussion

Our results show that the likelihood of death losses in transported pigs increases with increasing transport distance. The transport-related mortality ranged from 0.049% in pigs transported for distances below 50 km to 0.145% in pigs transported for distances exceeding 300 km. The impact of external air temperature on the transport-related mortality found in our study clearly shows that current transport practices fail to ensure the welfare of pigs transported under other than moderate weather. Particularly cold temperatures below –2 °C were associated with increased death losses in winter transport.

The death of animals during transport for slaughter is a major factor indicating the level of welfare in transported animals. Despite a decreasing tendency in the mortality of finisher pigs transported for slaughter in Europe, our study suggests that current transport conditions are not effective at ensuring the welfare of pigs during transport for longer distances and the protection of pigs against the negative impact of extreme ambient temperatures. Further research should focus on developing practical guidelines to improve the welfare of the pigs in transit accordingly.

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Comparison of selected plasma indices in pigs stunned with carbon dioxide and electric current

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Introduction

Council Regulation (EC) No. 1099/2009 on the protection of animals at the time of killing lays out a number of conditions to ensure animal welfare during slaughter and provides stunning methods applicable in EU member states. The most widespread ways of stunning pigs at the slaughterhouse are electrical and gas stunning (1). More than 30% of all slaughterhouses in the EU use carbon dioxide to stun pigs (2). Gas stunning has a number of advantages over electric stunning. Nevertheless, the application of CO_2 during the stunning of pigs has been the subject of much discussion. The objective of this study was to compare the physiological and biochemical events that occur in pigs after both, carbon dioxide anaesthesia and electrical stunning.

Materials and Methods

At lairage, pigs of same origin were randomly selected and divided into two groups. The first group of pigs (n = 20) was allocated to a dip-lift stunning system and exposed to 85–90% CO_2. The pigs were stunned in groups of four. In Group 2 (n = 20) head-only electrical stunning was carried out manually in a stunning box. An electric current of the following parameters was applied: input voltage 230 V, frequency 50 Hz, power input 4.3 A, stunning voltage 380 V, current intensity 1.3 A, frequency 400 Hz, time 2 s. Blood for laboratory analyses was collected from the blood draining from the thoracic stick about 5 seconds after bleeding started into 10 ml test tubes containing 100 µl heparin. Subsequently, centrifugation (800 x g, 10 min at 4 °C) was performed. After plasma separation, the samples were placed into cryotubes and stored at –85°C until analysis was performed about a week later. Samples were assayed to determine adrenalin, noradrenalin, plasma cortisol, ALT (alanine aminotransferase), AST (aspartate aminotransferase), cholesterol, glucose, lactate, LDH (lactate dehydrogenase), total protein and triglycerides. The results were analysed using the statistical package Unistat 5.1. (Unistat Ltd., GB). The data was subjected to a one-way ANOVA with the stunning method as the main effect (two levels: carbon dioxide and electric current).

Results

Highly significant differences ($p < 0.01$) were found between treatments (either gas or electrical stunning) in plasma cortisol (33.98 vs. 92.00 ng/ml), noradrenalin (799.30 vs. 388.10 nmol/l), total protein (91.30 vs. 83.42 g/l), glucose (9.83 vs. 7.48 mmol/l), lactate (12.94 vs. 19.68 mmol/l), AST (3.38 vs. 1.84 µkat/l) and ALT (1.66 vs. 1.22 µkat/l) levels. The results concerning adrenalin, cholesterol, LDH and triglycerides levels did not show significant differences between treatments.

Conclusions and Discussion

The secretion of cortisol and catecholamines is known to increase in response to many types of stressor. The release of adrenalin from the adrenal medulla is induced primarily by emotional stressors, though also by, for example, hypoglycaemia and immobilisation. In contrast, pain (and cold) do not activate adrenalin, but stimulate the release of noradrenalin from sympathetic terminals (3). Pigs stunned with carbon dioxide showed a level of noradrenalin more than twice as high as the second group ($p < 0.01$). The action of carbon dioxide evidently causes a significant increase in the level of this catecholamine in the blood in comparison with individuals stunned with an electric current. If the pigs stunned with CO_2 in this study showed a higher level of catecholamines in the blood plasma, the glucose content was also similarly increased ($p = 0.005$). In contrast, values for the content of lactate following stunning with gas were lower than those with the use of an electric current.

The results of the present study showed that stunning methods affect most of the blood parameters monitored. Significantly higher plasma levels of noradrenalin, ALT, AST, glucose and total protein were found in CO_2 stunned pigs, whereas significantly higher plasma levels of cortisol and lactate were found in electrically stunned pigs. When electric stunning is used, unconsciousness is instantaneous. High levels of stress hormone would have no effect on welfare because the pigs would be unconscious. In CO_2 stunning, the onset of unconsciousness is not instantaneous and further research is needed to determine if the massive release of noradrenalin occurs before or after the onset of unconsciousness.

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Animal welfare and production, are they compatible?

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Introduction

The animal welfare (AW) law of Sweden has high standards. Sows must always be kept free, even at farrowing; tails are un-docked; weaning is not allowed before 28 days (d) at an individual level; growth-promotors have been banned since 1986; and the space per animal is higher than in e.g. EU (Fig. 1) [1].

It has been argued that advances in AW have increased production costs compared to other countries. Therefore, the Board of Agriculture, the pig producers and the pig health organizations designed a trial in 10 selected pig herds that compared productivity and AW when deviating from the current AW legislation.

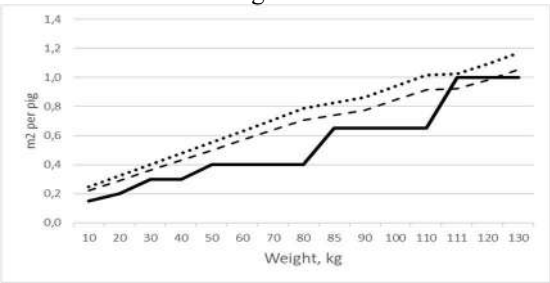

Figure 1. Area in AW law per pig in Sweden (dotted line) and in EU (black line). The demand in Sweden reduced with 10% as in **IV** and **V** of the study (dashed line).

Materials and Methods

Independent experts (the authors), evaluated the obtained results, but had not performed data recording, although given feedback on the study design.

Health and performance was compared between the previous year (Control; C) and the year of the trial (T) in 5 sub-parts: **I.** Decreased weaning age to 28 d on average (n=3); **II.** Confinement of sows ≤5d after start of farrowing (n=4); **III.** Confinement of sows ≤7d post weaning (n=3); Increased stocking density by 10% in weaner pens (**IV**, n=3) and in fattener pens (**V**, n=3).

Results

I. Piglets were weaned at 32.8±1.6d in C, and at 27.5±1.3d in T. Thus, 1.2±0.3 more piglets were weaned per sow and year in T ($p<0.001$). The weaning weight decreased from 9.3±0.2 kg in C to 8.7±0.4 in T ($p<0.001$), but the age at 30 kg was similar (81.4±4.4 in C and 78.4±4.6 in T; n.s.). The back fat of the sows did not differ between groups.

II. Unconfined sows weaned 26.2±1.6 piglets per year, compared to 26.0±2.6 piglets of confined sows (n.s.). No significant difference in piglet mortality was found.

III. When dry sows were confined after weaning, the hygiene turned poor and this part of the study was terminated due to the problems.

IV. Piglets in C reached 30 kg body weight at 78.2±3.6d and when the area per piglet was reduced with 10% (T) they reached 30 kg bw at 80.4±6.7d (n.s.) (DWG from weaning 478±44g vs 449±53g; $p=0.14$). Mortality was significantly higher in T than C (1.6% vs 1.1%, $p=0.02$).

V. DWG (863±54g vs 864±4g; n.s.), feed conversion (27.3 in both groups) and pathological lesions recorded at slaughter were similar for both groups.

Conclusions and Discussion

By reducing the length of the nursing period with almost 5 days, 1.2±0.3 more piglets were weaned per sow and year without recording significant side effects. However, as piglets are immature, weaning a few days earlier might have a negative impact although data in this study did not show that. In Sweden sows are kept in intact groups at farrowing, therefore an average group weaning age of 28d was judged acceptable. An average group weaning age of 28d imply that no pig will be less than 25d at weaning, which is important as the immune system mature significantly from 3 to 4 weeks of age [2].

No increased production nor decreased piglet mortality was found when sows were confined ≤5d after farrowing started. Thus, confinement of sows at farrowing was found irrelevant. However, the farrowing pens in Sweden must be at least 6 m^2.

Sows riding at heat 3-5d post weaning is a serious probelm[3]. However, the solid floor in the eating pens where sows were captured for ≤7d post weaning were for hygienic reasons not suited to prevent riding.

No significant effect of increasing stocking density in weaners or fatteners was found. However, an increasing in density with 10% will increased risk of disease transmission with 21%. Thus, it was concluded that an increase in density could be accepted in piglet producing herds and in integrated herds with old older buildings, as their productivity have increased over time due to an increased number of piglets produced. In contrast, this cannot be accepted for specialized fattening herds purchasing growers nor to build new stables designed for an increased density.

The conclusions of this study is at present discussed in Sweden, and a decreased nursing period (**II**) has been allowed in farms that adopt a special AW scheme.

Acknowledgments

We thank the stakeholder organizations. The study was financed by the Board of Agriculture.

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Health, animal welfare and working environment in a new farrowing pen

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Introduction

Many tasks in pig stables are laborious and repetitive[1]. Reducing piglet mortality is one of the most important jobs for the staff, often demanding a large part of the working time[2]. The present study aimed to improve piglet welfare and reduce workload by designing a pen for free-farrowing sows aiming to influence the behavior of the sows to simplify suckling for piglets and work for staff. As both sows and litter sizes have increased with time, the farrowing pen was enlarged by placing the piglet hut outside a traditional pen, creating more space for both sow and piglets. The new pen was 33% larger than current minimal regulations in Sweden (6 m^2) and 120% larger than applied in many EU countries (3.6 m^2).

Materials and Methods

The farrowing pen was 8 m^2, of which the sow had access to 6.85 m^2. The lying area of 4.1 m^2 had solid floor, and 2.75 m^2 was drained. To improve hygiene, feed and water cups were placed over the drained floor (Figure 1).
The piglet hut was 1.15 m^2 and placed parallel to the presumed lying area of the sow. The idea was to attract sows to lean their udders to the heat from the hut and facilitate suckling (Figure 1).
Tails were un-docked, and no routine administration of antibiotics was given. Two pens were filmed for 24 hours on the day before farrowing, farrowing day and days 3, 7, 14 and 21 after farrowing. One camera filmed the pen, and another filmed the piglet hut. Behavior of sow and piglets, as well as work of staff, were recorded and the staff (n=3) replied to an inquiry.

Results

During 2019, sows delivered 14.0 liveborn piglets (Ntnl mean 14.0). The preweaning mortality was 16.2% (Ntnl mean 16.9%). Thus 11.8 piglets were weaned at an age of 31 days and a weight of 10.75 kg (DWG *- weaning 295 g/d), and no piglet was treated for diarrhoea (Ntnl mean 11.6 piglets at 32.8 days weighing 8.2 kg; DWG *- weaning 204 g/d).
During the first week of life, all suckling took place at the intended location (Figure 1) where the sows also spent all their time and directed their udders to the piglet hut most of the time. Thereafter, 16-22% of the sow activities took place at the drained floor.
Initially, piglets spent most of the time in the hut, but already on the first day of life they knew where the dunging area (drained floor) was. Nose to nose interactions between sow and piglets were frequent on day 3 and day 7 after birth, but then decreased.
The suckling time was 10.5 hours the farrowing day but decreased to around 2 hours on Day 7 and around 1.5 hours on Day 21. During days 3, 7, 14 and 21, piglets suckled at 27.6±2.1 times per day during 4.4±1.0 minutes per suckling. Piglets rested or slept around 17.5 hours and practiced other activities around 3 hours per day from Day 3, and they made playful behaviors (rushing, rotations and jumping) from Day 7. They had access to supplementary feed and iron-enriched peat in the piglet hut from Day 3. Intake of these began Day 14 coinciding with feeding.

Figure 1. Sow with the udder to the piglet hut and piglets suckling from there while administrating shavings with the Perspex ceiling partly removed. The drained floor to the left.

Interactions between sows and staff were peaceful. The pens were visited 3.4±0.9 times per day for care (cleaning, providing, straw *etc.*), examination of pigs and piglet feeding. The working time per pen was 14 minutes the farrowing day, 8 minutes on Day 3 and 2.5±1.2 minutes per day thereafter. Of this time, 1.9±0.9 minutes was used for care. The time for piglet feeding was 8.8±2.4 seconds per day from Day 3. The staff made 15.5±6.8 different tasks with 14.4±7.4 different working positions per day, 2 with twisted backs (0.7%) and 282 with straight backs (99.3%).
According to the inquiry, care and treatment of pigs was simplified because it was easy to see and catch the pigs. Large-scale cleaning between batches was also easier.

Conclusions and discussion

Sows and piglets were given access to a larger area than in general and performed well in the farrowing pens. Piglets were aided to suckle due to the location of the hut in parallel to the udder, which also protected them from being accidently crushed by the dam. Piglets had a high DWG from birth to weaning, mirroring a good start in life. The workload was low with ergonomic working positions. Essential tasks were easy to carry out, creating time to focus on the pigs and their well-being.

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Inspiring a verified culture of care post identification of abuse on an undercover video using a baseline assessment of expressed competencies compared to post training measurement of expressed competencies

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Introduction

Sometimes the need to change human behavior is determined from internal evaluation, and sometimes the need to change is uncovered and stimulated from the outside. This case study presents a company's training response to an undercover video taken by a swine farm employee simultaneously working for Mercy for Animals. The video identified unacceptable behavior outside of company protocol on a sow farm (Farm A). Two animal handling actions captured on video were animal abuse which resulted in immediate dismissal of the accountable employees. This case study details the training plan created for remaining employees potentially influenced by the expression of these unacceptable behaviors, and shares expressed competency results of employees after re-training compared with the baseline expressed competency results recorded the day of the release of the undercover video.

Materials and Methods

Immediate Actions and Baseline

The same day of the undercover video release all pigs on site were assessed by a 3rd party veterinarian to ensure all pigs were safe and to help assess needed actions. A medical record outlining needed change was created. The system's production team and veterinarian discussed with the 3rd party veterinarian and agreed on an action and training plan.

Training Plan

This targeted response plan included first a Farm A specific re-training plan on animal handling followed by some lesson specific topics that worked to address missing expressed competencies. Farm A's successful completion would be followed by a system-wide re-training plan on animal handling. The program components per learning experience includes online training, graphical SOP's and in-barn verification which employed the *See it. Do it. Teach it.* methodology[1,2,4,5,6,9]. Training delivery occurred via learning experiences delivered on PATP's online learning management software. Simultaneously, an internal and external verification plan was detailed and executed post re-training to measure expressed competency results using the lesson's In-Barn Verification Checklists as guidance documents All training accomplishments were recorded on PATP's online learning management software and a transcript of learner accomplishment created per learner[7].

See it.

Farm A chose to administer most videos and quizzes as a group and have a discussion after each lesson on skills they needed to express differently on farm. Learners were informed prior to the videos that they needed to learn the topics well enough that they could teach it back to a verifier.

Do it.

Then the learners continued to complete skills which were manager or mentor observed until verified. If desired, pictorial SOP's were available for review in English and Spanish.

Teach it.

Internal verification included having individuals teach what they learned back to an In-barn Verifier. In-barn Verifiers presented themselves to each employee as "their student" and encouraged them to "pretend I am a new employee and teach me how to do this task." While they spent most of the verification experience listening, verifiers utilized the In-barn Verification Checklists to make sure all key competencies were taught back successfully according to the farm protocol and asked about a key competency if it was not brought up specifically. When individuals successfully passed their In-barn Verifications, Verifiers marked this part of the learning experience complete on their transcript.

Results

The baseline expressed competencies for the training curriculum chosen was at a 26% competency expression rate on farm for chosen handling competencies. Post external verification of training, staff averaged a 95% handing competency expression rate. This is a 68-percentage point difference and a 268.8% increase in handling competency expression on farm. Retraining throughout the entire system is occurring throughout Fall and Winter of 2018/19. External verification of other farms occurs in December 2018 and January 2019, and results will be reported in this presentation.

Conclusion and Discussion

Using this methodology[1,2,4,5,6,9] has effectively improved competency expression in other studies from a 59% level of competency expression to an 85% competency expression level[8]. This case study also achieved improved levels of expressed competency.

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Prevalence and distribution of foot lesions in Italian culled sows

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Introduction

Claw and foot lesions are very common diseases, with a prevalence ranging from 88% to 100% (1). These lesions, in association with lameness, are considered the second largest reason for the early culling of sows on farm (2). Therefore, toe health must be considered not only a welfare concern but also an important economic issue for the pig sector. Locomotor disorders have a multifactorial ethiopatogenesis, including nutrition, genetic, management strategies as well as housing systems with particular regard to the flooring type (3). Despite the relevance of the problem at international level, in Italy too little information is available on its prevalence and the related risk factors. In order to improve housing conditions and the welfare of sows at farm level, the aim of this study was to collect at slaughterhouse data on the prevalence and the type of foot lesions in Italian culled sows from different farms and to analyse possible differences between them.

Materials and Methods

The present study was carried out in a slaughterhouse located in the North of Italy. Overall, 1704 feet (3408 claws and 3408 dewclaws) of 426 culled sows from 16 different farms were evaluated after stunning and bleeding. Lesions of all the parts of the hoof were examined and these include: heel overgrowth and erosion (HOE), heel-sole crack (HSC), sole fracture (SF), horizontal cracked wall (CWH), vertical cracked wall (CWV), white line damage (WL), claw and dewclaw coronary band damage (CB, DCCB) and claw and dewclaw excessive length (T, DC). Differences between farms were assessed using Chi-square test (GraphPad® Software Inc. San Diego, CA, USA), taking into account only farms with greater than or equal to 20 sows evaluated (8 herds).

Results

The overall prevalence of foot lesions was 98.6%, with 420 sows with at least one digit affected. The prevalence of individual lesions is shown in Table 1. The most common foot lesions were CWH (79.8%), WL (68.3%) and DC (50.2%). Lateral digits were more frequently affected than medial digits in all four feet (29.5% vs 20.1%, respectively) and the hind feet had more injuries than the front feet (29.4% vs 20.1%, respectively). Significant differences between farms were found for FS, CWV, CB, DCCB ($P<0.05$) and for all other types of lesions ($P<0.01$).

Table 1. Number and prevalence of sows with at least one digit affected.

Lesion	Number	%	95% CI*
HOE	109	25,6	21,4-29,7
HSC	352	35,7	31,2-40,2
SF	99	23,2	19,2-27,3
CWH	340	79,8	76,0-83,6
CWV	189	44,4	39,6-49,1
WL	291	68,3	63,9-72,7
CB	128	30,0	25,7-34,4
DCCB	19	4,5	2,5-6,4
T	80	18,8	15,1-22,5
DC	214	50,2	45,5-55,0
Tot.	420	98,60	97,5-99,7

*CI: confidence interval.

Conclusions and Discussion

In accordance with literature this study showed a very high prevalence of sows with at least one digit affected (98.6%). In particular, lesions were more frequent on the lateral claws of the hind feet (4, 5). It could be explained by the wetter conditions of the rear claws because of urination, that let to soften the hooves and diminish the horn ability to resist pressure. Furthermore, lateral digits bear the weight of almost the 78% of the total load and have a larger area in contact with the ground than the medial digits, thus a higher prevalence of lesions could be expected (3). The significant differences between farms could suggest the influence of some factors related to farm housing and structures (with particular regard to the flooring characteristics) on the development of foot lesions. Further studies are necessary to identify risk factors related to claw lesions, to deepen the association between foot injuries and floor properties (slipperiness, abrasiveness, void ratio) in order to reduce the culling rate of sows, improve foot health and thus animal welfare at farm level.

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Foot lesions in Italian heavy pigs: preliminary results

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Introduction

The Italian pig sector is mainly focused on the production of heavy pigs used for traditional Protected Designation of Origin products. Pigs are slaughtered at 8-9 months of age when they reach a live weight of 150-170 kg (1). It has been demonstrated that as bodyweight increase, pressure on the weightbearing areas of the feet increases (2). As a result, fattening pigs are often affected by claw and limb lesions, reaching an overall foot lesion prevalence of 93.8% (3). Pig longevity has a great impact on the productivity and economics of the swine industry and, as one of the main reasons for premature culling, lameness can shorten their lifespan (4). Therefore, the aim of this study was to collect data on the prevalence and the type of foot lesions in Italian heavy pigs. In addition, deepening the associations between different farms and the prevalence of foot lesions could help in identifying risk factors related to farm housing and structures, in order to improve animal welfare.

Materials and Methods

The present study was carried out in a large specialist pig abattoir in the North of Italy. A total of 1741 pigs from 19 farms located in Northern Italy were evaluated. Overall, lesions from 3482 front feet (6964 claws and 6964 dewclaws) were recorded after stunning and bleeding. Due to the structural features of the abattoir it was possible to examine only the front feet. Heel overgrowth and erosion (HOE), heel-sole crack (HSC), sole fracture (SF), horizontal cracked wall (CWH), vertical cracked wall (CWV), white line damage (WL) claw and dewclaw coronary band damage (CB, DCCB) and claw and dewclaw excessive length (T, DC) were considered. Differences between farms were calculated using a Chi-square test (GraphPad® Software Inc. San Diego, CA, USA), taking into account only farms with more than 20 pigs evaluated (18 herds).

Results

The prevalence of foot lesions of the 1741 pigs is shown in Table 1. The overall prevalence of claw injuries was 41.5%. WL was found to be the most frequent lesion (21.9%) whereas HOE and CWH were only occasionally found (7.2% and 4.1% respectively). Significant differences between farms were found for HOE ($P<0.001$), HSC ($P<0.001$), FS ($P<0.01$), CWV ($P<0.01$), CWH ($P<0.001$) and WL ($P<0.001$).

Table 1. Number and prevalence of pigs with at least one digit affected.

Lesion	Number	%	95% CI*
HOE	125	7,2	4,7-9,7
HSC	58	3,3	1,6-5,0
FS	33	1,9	0,6-3,2
CWH	71	4,1	2,2-6,0
CWV	43	2,5	1,0-4,0
WL	382	21,9	18,0-25,8
CB	1	0,1	0-0,4
DCCB	1	0,1	0-0,4
T	5	0,3	0-0,8
DC	1	0,1	0-0,4
Tot.	720	41,5	36,8-46,2

*CI: confidence interval.

Conclusions and Discussion

The overall prevalence of foot lesions in the current survey was 41.5%, very different from studies that reported percenteges higher than 90% (3). This outcame may be influenced by the observation of front feet only, since literature reveal higher prevalences of claw lesions on rear feet (6). In addiction, swine weight category should be taken into account as an important variable. Nevertheless data on the most frequent foot lesions were in accordance with literature (3), in fact white line damage (21.9%) and heel overgrowth and erosion (7.2%) were found to be the most common injuries. Previous studies reported that sows with WL lesions were more likely to develop lameness, which is considered an important animal welfare issue (5). Mouttotou et al. (1999) showed a significant association between partially slatted floors and a higher prevalence of heel erosions, white line damages and wall separations. In this regard further studies are necessary to identify flooring-related risk factors associated to the development of foot lesions, for improving pig health and welfare at farm level.

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Using machine learning to determine sound-based signal pattern at different temperatures in nursery phase swine

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Introduction

Pork is the most consumed meat in the world. The main goal of the market is to achieve maximum productivity using the lowest possible financial resources, which directly affects the life quality of the animal. Due to the growing concern about the quality of food and ethics involved in animal production by consumers, investors, and importers, this study proposes a non-invasive analysis, using microphone, by applying machine learning techniques, which can generate accurate decision-making based on behavior responses.

Materials and Methods

For three days the authors collected data about vocalization of Landrace X Large-White swine group (composite by six pigs) and environmental noise. The animals were located under air-conditioned shed in facilities from University of Illinois at Urbana-Champaign (EUA) and the signals were collected by a microphone. The first day temperature was 27º C, while second day had a high graduation from 27º C to 34º C. Finally, the third day had an temperature increase from 24º C to 34ºC. The collected data were filtered by low-pass and high-pass filter, then it was submitted to the Fast Fourier Transform. Signal energy, maximum and minimum amplitude, maximum, minimum and median intensity, pitch and harmonics were the parameters extracted for this study. In order to determine the standards, these parameters were submitted to a machine learning routine in MATLAB environment, called Neural Net Clustering, using ten neurons. It was found that some data groups presented some similarities (Figure 1), but it was considered pattern the groups with at least fifty five audios (2.5% of total).

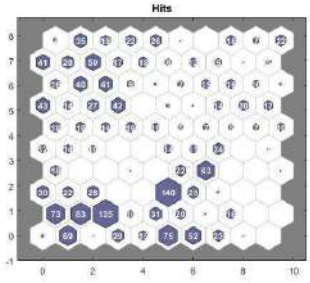

Figure 1. Result of Neural Net Clustering

Results

Seven patterns were selected in all three days analysis. These patterns have differences in temperature and day period in which each data group was collected, indicating different levels of animals activities over the day and under different temperatures.

Conclusions and Discussion

The minimum amplitude was not indicative about any especific condition. All patterns presented the same number, while the energy of signal presented different medians among seven patterns, but it was not possible to identify a clear differentiation among different temperature conditions (sub-normal, normal, and above normal). Pitch and harmonics had differences under different temperatures. The seventh pattern presented the lowest pitch average and had 79% of data collected under hot temperature condition (about 31º Celsius). The harmonics were less when the patterns had a data collected in hot or moderate temperature conditions, agreeing with pitch results. Pitch and harmonics give the signal identity. Different temperatures have different responses because differents activities occur in each condition. The next step will be to evaluate which activities occur in sub-normal, normal, and above normal temperature. The maximum median intensity was less in pattern two, where the temperature was above 31º Celsius. High temperatures force animals to execute less activities to produce less energy and thus to catch up termal confort zone. This methodology of finding patterns for signals parameters can be improved. The signals used on this research had many noises that were constant and similar to swine vocalization, so it coudn't be substracted. Collecting new data, using the same methodology, the pattern will be more accurate and could identify moments with different temperatures and the activities that are ocurring at each moment.

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Transporting weaned piglets in winter: the effects of density on weight and salivary cortisol

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Introduction

Increased pig production has increased the demand for weaned piglets for the nursery. However, some regions are not self-sufficient in their production of piglets. Transport is one of the main factors affecting welfare in pig production since the transfer of animals between the different stages of production and from farms to slaughterhouses generates stress, which negatively affects health and productivity (1). This research aimed to study differences in the weight and physiological indicators of the welfare of weaned piglets transported at different densities, to the nursery phase, during winter.

Materials and Methods

Were used 480 animals, from the same sow farm, that were born and weaned in the same week, with an approximate age of 28 days. The piglets were transported in trucks to the nursery facility, during winter, for approximately 14 h in six groups of 80 piglets at different densities: 0.091 m^2/piglet, 0.077 m^2/piglet, 0.064 m^2/piglet, 0.057 m^2/piglet, 0.052 m^2/piglet, and 0.047 m^2/piglet. The welfare of the six groups was evaluated by measuring changes in salivary cortisol, and weight before and after transport. To the salivary cortisol analysis, samples were collected from a pool of piglets from each group by allowing them to chew cotton ropes until they moistened. The data were analyzed using the GLM procedure of the statistical package SAS to conduct an analysis of variance.

Results

The lowest density group (0,091 m^2/piglet) showed the greatest mean weight loss of 0.469 kg, and none of the other groups were significantly different (Table 1). An increase in salivary cortisol was observed after transport (Table 2), which decreased after a few days of housing.

Table 1. Mean weight (kg) of weaned piglets before and after transportation to the nursery at different densities (m2/piglet).

Group	Density	Weight before	Weight after	Difference
A	0,091	7,858	7,416	-0,469 b
B	0,077	8,191	8,119	-0,061 a
C	0,064	8,332	8,157	-0,153 a
D	0,057	7,649	7,613	-0,073 a
E	0,052	7,970	7,867	-0,112 a
F	0,047	8,402	8,172	-0,202 a

Different letters in the weight difference column represent significant differences at $P < 0.05$.

Table 2. Levels of salivary cortisol (ng/ml) on the day before transportation (M1), when piglets were housed at the nursery (M2) and on the 3rd (M3), 4th (M4) and 5th (M5) days of the piglets being housed at the nursery

Group	M1	M2	M3	M4	M5
A	0,49	1,6	1,8	0,1	0,5
B	0,49	1,8	2,1	0,3	0,4
C	0,49	2,7	0,5	0,5	<0,1
D	0,49	3,1	0,2	<0,1	<0,1
E	0,49	1,6	<0,1	<0,1	<0,1
F	0,49	19,5	0,1	0,5	<0,1

Conclusions and Discussion

Transport density at winter was able to influence weight loss since piglets transported at a low density showed greater weight loss. It is likely that the larger spaces between piglets make it difficult to stay in condition, due to the lack of support that the most juxtaposed contact with other piglets provides. This causes greater imbalance during truck movement, requiring more muscle effort to remain stationary, whether stationary or lying down, leading to greater fatigue and consequently greater weight loss. As well as helping with thermoregulation, it is also economically advantageous because more piglets can be transported in one truck. The increase in cortisol after transport and stabilization at the other collection points clearly demonstrates that transportation is a stressful time for the piglet. This conclusion is supported by the fact that the salivary cortisol collection method excludes two stressors: animal containment and venipuncture. It is possible to transport piglets at higher densities during winter because it does not affect animal welfare when compared to lower densities.

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Effect of Betaine and Isoquinoline Alkaloids supplementation in pigs under Heat Stress conditions

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Introduction

Heat stress (HS) compromises efficient pork production by reducing growth performance, reproductive performance, meat quality (St-Pierre, et al., 2003; Wegner, et al., 2016). The mechanism is in part due to increased oxidative stress and inflammation, particularly within the gastrointestinal tract (GIT) (Cottrell, et al., 2015). The damage to the GIT can be ameliorated with anti-oxidants (Liu et al, 2016), demonstrating that nutritional strategies may be a useful tool for the management of HS. The effect of isoquinoline alkaloids (IQ) extracted from *Macleaya cordata*, and betaine on symptoms of HS and GIT integrity were studied. IQ have anti-inflammatory properties and protective effects on gastrointestinal integrity in pigs (Artuso-Ponte, et al., 2015; Lee, et al., 2016). And betaine is both a methyl donor and osmolyte. Betaine supplementation reduced oxidative stress, prevented tissues damage and anti-inflammation (Gabler, et al., 2013; Zhang, et al., 2014). The objective of the present study was to investigate the effect of IQ and betaine supplementation on ameliorating HS response in pigs.

Materials and methods

Fifty female Large White x Landrace pigs (av. 27.3 kg) were acclimated one of the following diets: control (CON) (standard grower diet), Betaine (BET, + 1 g Betaine /kg feed), Isoquinoline Alkaloids (IQ, + 150 g/ton botanical feed additive) for 14 days under thermoneutral conditions (TN, 20^0C). After adaptation period, pigs were moved to climate rooms and housed under TN or cyclic heat-stress (8 h 35^0C, 16h 28^0C/d) for 3 days. During the climate challenge respiration rate, rectal temperature and skin temperature were measured every 2 hours. On day 3 of the climate challenge pigs were euthanised, blood collected, transepithelial electrical resistance (TEER) and macromolecule permeability quantified with Ussing chambers. All data was statistically analysed using an ANOVA with Duncan post-hoc tests using Genstat v18.

Results

HS increased respiration rate (RR), rectal temperature (RT) and skin temperature (ST) of pigs compared to TN. However, both BET and IQ reduced RR compared to CON (169^a, 150^b and 158^b breaths/min for CON, BET and IQ respectively, P=0.013) and RT (40.1^a, 39.8^b and 39.9^b, P=0.001). Heat stress reduced plasma creatinine, lactate, urea and thyroid hormone (T_3 and T_4), however no effects of HS on cortisol or triglycerides were observed. Furthermore, no effects of diet or interactions with HS were observed on metabolites or hormones. BET improved total antioxidant capacity (TAO) in comparison to CON and IQ independently of HS (1.34^a, 1.64^b and 1.19^a mM Trolox, P=0.010). IQ diet increased ileum TEER compared to BET and CON under TN, but not HS conditions (vs and 34.8^a, 37.2^a and 60.8^b, P=0.05). The increase in colon permeability with HS was ameliorated by both BET and IQ (1.21^a, 0.53^b and 0.77^b μg/min.cm^2, P=0.057).

Conclusion

IQ and betaine supplementation ameliorated the effects of HS in the growing pig and protected against damage to the GIT. Therefore, they could be used as a nutritional strategy to reduce the negative impact of HS on gut health and performance.

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