SWINE HEALTH AND PRODUCTION:

UPDATING, INNOVATION AND TECHNOLOGY

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Foreword

It is with great pleasure that I write the foreword to this book entitled "Health and Swine Production: Updating, Innovation and Technology". As evidenced in the title, this set of scientific texts brings practical and relevant information, supporting the leaders in pig farming to make evidence-based decisions. These decisions will naturally result in a positive impact on the health, well-being, and productivity of pig herds. The texts were written by global leaders in pig health, epidemiology, and productivity, summarizing the content of their respective explanations at the FarmaTalks® Swine 2020 Conference. The conference brought together more than 6,200 participants, representing much of the global pig industry. Farmabase, the organizer of FarmaTalks®, ran the non-profit program, focusing on the diffusion of scientific content of practical value for veterinarians, animal scientists, and pig producers. Sponsors of the event donated food (rather than monetary values) to charities. Thus, the high scientific value of this material, and the nobility of the organization of the event, generating and distributing knowledge in a beneficent manner, make this work an authentic innovation, raising the standards of global swine conferences. I wish you all an enjoyable reading. I suggest reading with a pen and paper because it has a lot of rich and innovative information that you will want to write down!

Sincerely,

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Publishers

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Veterinarian graduated from the Federal University of Paraná – campus of Palotina (2006). Specialized in Hygiene and Processing of Products of Animal Origin by UFPR - campus of Palotina (2009), has an MBA in Strategic Management of Agribusiness by Fundação Getúlio Vargas (FGV) (2013). Master of Veterinary Medicine in the area of Veterinary Clinical Medicine at the School of Veterinary Medicine and Animal Science (FMVZ/UNESP), campus of Botucatu (2015-2016). Ph.D. student at the School of Agricultural and Veterinary Sciences, São Paulo State University, Jaboticabal-SP, Brazil. He worked in the agroindustry for seven years between 2006 and 2013, as manager of agriculture and development, joined the veterinary pharmaceutical industry in 2014 at Farmabase Animal Health, where he is currently Technical Manager for Swine and Poultry.

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Summary

01

Respiratory Health

Mycoplasma hyopneumoniae infections in pigs: control or eradication?

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Mycoplasma hyopneumoniae (*M. hyopneumoniae*) is the primary pathogen of enzootic pneumonia, a chronic respiratory disease in pigs, and one of the primary agents involved in the porcine respiratory disease complex (PRDC). Infections occur worldwide and cause major economic losses to the pig industry. Losses are mainly due to costs for treatment and vaccination, decreased performance and increased mortality in case of concurrent infections. The organism is primarily found on the mucosal surface of the trachea, bronchi, and bronchioles, and close contact between infected and susceptible pigs is the main route of transmission.

Improvement of the management practices is primordial in the control of *M. hyopneumoniae* infections. These include all-in/all-out production, proper gilt acclimation, stabilizing herd immunity, maintaining optimal stocking densities, prevention of other respiratory diseases, and optimal housing and climatic conditions. The introduction of *M. hyopneumoniae* naïve gilts into endemically infected farms represents a significant challenge for the incoming gilts and for the recipient sows. Naïve gilts may be exposed to positive sows, may become infected, and subsequently transmit the pathogen to the newborn piglets. The sows of the recipient herd can potentially get (re)infected, generating infection imbalances in the herd. To prevent these problems, incoming gilts should be vaccinated properly before they join the sow population. Another possibility is to purposefully expose incoming gilts at a young age to *M. hyopneumoniae*, aiming gilts to recover and become immune prior to entering the sow farm, and to no longer shedding the bacterium. Preliminary studies have shown that selection for disease resistance may be helpful in the control of *M. hyopneumoniae* infections, although positive effects are not consistent. Strategic medication in chronically infected herds has also been used to control *M. hyopneumoniae* infections. Long-term and/or preventive medication however should be discouraged because of the increased risk of antimicrobial resistance development. The currently available vaccines reduce clinical signs and lung lesions, improve performance, reduce the number of organisms in the respiratory tract and decrease the infection level in a herd. Therefore, they are often cost-efficient. However,

vaccination confers only a limited reduction of the transmission ratio of *M. hyopneumoniae*. Research should be continued to develop new vaccines that confer protective immunity and reduce transmission.

Successful elimination of *M. hyopneumoniae* from swine herds has been reported and several protocols have been developed. Elimination of *M. hyopneumoniae* from commercial herds, either alone or in combination with the elimination of other pathogens, might be a good option for some farms. Further research to refine protocols for practicality and application in combination with other disease elimination programs is required.

Further reading: Book Mycoplasmas in Swine:
https://www.acco.be/en/items/9789463797962/Mycoplasmas-in-Swine

Porcine Circoviruses in 2020: What's new?

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Introduccion

Porcine circoviruses (PCVs) are small DNA viruses and, so far, have four representatives, PCV-1, PCV-2, PCV-3 and, tentatively, PCV-4. PCV-1 is known as non-pathogenic for pigs, while PCV-2 has been associated with several conditions known as porcine circovirus diseases (PCVDs). PCVDs include PCV-2 systemic disease (PCV-2-SD), PCV-2 reproductive disease (PCV-2-RD), porcine dermatitis and nephropathy syndrome (PDNS), and PCV-2 subclinical infection (PCV-2-SI). The PCV-2-SI is probably the cause of the greatest economical losses for the pig industry, because of the virus effect on average daily weight gain. In 2015, PCV-3 was firstly described in sows displaying reproductive failure and PDNS, as well as in pigs with multisystemic inflammation. Since then, many other descriptions of the virus presence came up from pigs displaying several diseases and even in healthy animals. PCV-4 is the newest tentative member of the *Circoviridae* family and was described in pigs displaying respiratory and digestive clinical signs as well as PDNS. It is already known that PCV-1, PCV-2 and PCV-3 are ubiquitous pathogens, while PCV-4 has been only detected in China so far.

Porcine circovirus 2 (PCV-2)

PCV-2-SD is a multifactorial process that can be efficiently controlled by means of PCV-2 vaccination. Before the advent of immunization products, the disease was tried to be controlled by means of ameliorating the effect of infectious and non-infectious factors triggering the clinical condition. However, by mid-2000 few vaccines appeared in the market of few countries and their effect on counteracting the economic losses due to PCV-2-SD were outstanding. Moreover, since this disease caused immunosuppression in pigs, vaccination helped controlling other polymicrobial processes in which PCV-2 was involved.

Most of the vaccines commercialized worldwide today are based on genotype PCV-2a (especially in Europe and Americas), although new products based on other genotypes are becoming available. From an experimental point of view, these PCV-2a based vaccines can counteract the infection effects of PCV-2b and PCV-2d, but it is not really known if this protection is equal independent of the genotype. Although in recent years some cases of PCV-2-SD have been diagnosed despite vaccination in piglets, this scenario is believed to be due to maladjustments of the vaccine program rather than a lack of efficacy of currently commercialized vaccines.

Who should be vaccinated against PCV-2?

Vaccination of sows might have two potential objectives: 1) to prevent porcine circovirus diseases (PCVDs) of the offspring, or 2) to protect against PCV-2-reproductive disease (PCV-2-RD). In the first case, vaccination should take place at late gestation, as it is recommended by the manufacturers of the PCV-2 vaccines intended for sows. If the objective it to prevent PCV-2-RD, vaccination might be applied before mating, being at the lactating period or at weaning for 1st parity or older sows, or during the acclimatization in gilts.

A second possibility would be to select piglet vaccination as the way to control PCVDs in the farm; in fact, this is the most common practice by far. It is known that control of PCV-2-SD in affected farms is quicker if piglet (instead of sow) vaccination is used, observing a positive effect in the very first vaccinated batch. The main reason is that vaccine applied in pigs is able to elicit protective immune responses in the animal that subsequently suffer from the disease.

A third option is to vaccinate both sows and piglets. There are several reports on the benefits of this schedule at productive and virological levels. It presumably joints the benefits of controlling PCVD in a "continuous protection" fashion since it provides strong herd immunity by vaccination sows/gilts, and protects piglets against the development of PCV-2-SD and ameliorates the outcome of PCV-2-SI. Repeated sow vaccination by cycle should also potentially benefit the reproductive outcome. In this double vaccination scenario is important to take into account the putative interference of maternally derived immunity (MDI) upon PCV-2 vaccine efficacy in piglets, since colostrum intake provides higher amounts of PCV-2 antibodies. It is true that the levels of maternally derived antibodies (MDA) must be very high in order to jeopardize the effects of PCV-2 vaccination in piglets, at least with the so far tested vaccines. It would be interesting to assess if this is true for all vaccines in the market. This situation is obviously linked with the timing of piglet vaccination.

PCV-2 vaccination in a changing epidemiological scenario

During last years, the high vaccination pressure exerted in the world pig population has implied a change in the PCV-2 infection epidemiology. It has been observed that after such repeated vaccination, viral loads diminish over time, to the point, in some cases, with no detection of circulation evidence in pigs. This may imply certain batches of pigs reaching seronegative at slaughter age. In principle, is very positive since we are almost eliminating the effects of the virus on growth, but the situation may be different for those animals that will be selected as replacements (gilts and boars). Although with low prevalence, PCV-2 circulates in the breeding stock, and the introduction of naïve gilts into the system increases the likelihood of infection of these animals and the perpetuation of PCV-2 within the sow-herd. Under such scenario, the probability of infection during gestation (mainly of gilts) is higher, as well as the proportion of viremic-born piglets and early infection in the offspring. In turn, it may happen that we vaccinate already infected animals. Although from an experimental point of view, PCV-2 viremic pigs vaccinated against PCV-2 are able to cope with the infection, and able decrease viremia and histopathological lesions compared to a viremic non-vaccinated group, efficacy under field conditions may be variable.

Does "vaccination failure" occur?

During last few years it has been noticed an increase of PCV-2-SD diagnoses in farms with vaccinated piglets; the terminology "vaccination failure" has been used to designate those situations. What probably happens here is that vaccination at weaning might not provide sufficient time to develop vaccine-elicited immune response before natural infection and a proportion of animals may develop PCV-2-SD and not just a PCV-2-SI. Recommendations in this case: 1) perform sow vaccination, trying to delay natural PCV-2 infection; or 2) earlier PCV-2 vaccination (i.e., at 10-15 days of life). This latter option should be coupled with serological analyses indicating low antibody values at the time of vaccination. It is nowadays believed that "vaccination failure" scenarios (i.e., unequivocal diagnosis of PCV-2-SD in vaccinated pigs) are mostly associated with an inadequate management of the vaccine (conservation, dose applied, etc.) and timing of application (too early – potential interference with maternally derived immunity or at the time of early infections, too late – too close to natural infection, or in diseased animals – i.e., PRRSV viremia). Looking at the major causes of the so-called "vaccination failure", it is more a "human failure" rather a vaccine efficacy problem. If putative "vaccination failure" will occur in the future due to PCV-2 escape mutants is still to be determined.

Porcine circovirus 3 (PCV-3)

PCV-3 is an "old" recently discovered virus that is widespread in both domestic pigs and wild boar and has been found in several non-Suidae species. If these species are fully susceptible to the infection and play a role in the epidemiology of the virus is still to be determined.

PCV-3 can be found at all ages in domestic pig and few animals may suffer from persistent infections. The virus has been found in several clinical and pathological conditions, but a definitive proof of its pathogenicity is still lacking. Only recent studies using *in situ* hybridization have given a clue regarding disease causality, since PCV-3 DNA was found in inflammatory lesions of sick animals. How frequent is the disease caused by PCV-3 and under which conditions it occurs are still important questions to be answered.

The lack of virus isolates readily available to date to develop an animal model makes difficult the progress towards the generation of basic knowledge on PCV-3 pathogenesis and immunity. It is very likely that these aspects will be sorted out soon in the future, but they will depend on the research effort dedicated to this new pathogenic agent in the next few years. Moreover, depending on the demonstrated impact of PCV-3 on pig health, putative vaccine development might be developed.

Porcine circovirus 4 (PCV-4)

PCV-4 still represents a big question mark for the swine industry. To date, it has only been found in China in a couple of farms, in animal displaying lesions compatible with PDNS and reproductive disorders. If this virus was found there by chance or it is related with disease causality is currently unknown. Recently, a study performed in Italy and Spain using a limited number of pig samples was unable to find PCV-4 genome in them. Therefore, the distribution of the virus all over the world is also another unknown.

Conclusions

Among porcine circoviruses, PCV-2 is still the most important one from economically point of view as well as the only one with an unequivocal association with disease. Therefore, vaccination against this virus is an important element for porcine health management in modern farms.

PCV-2 vaccines still represent the best option for controlling PCVDs worldwide. However, the high vaccination pressure exerted in the last 10 years has implied a change in the epidemiology of this viral infection, fact that should be counteracted by determining the best vaccination timing of the

animals. Therefore, monitoring of PCV-2 infection is becoming a corner-stone for PCVD prevention and control. Moreover, the classical diagnostic approach of PCV-2-SD (histopathology and viral detection in tissues) is increasing in the framework of the suspected "vaccination failure" scenarios.

On the other hand, PCV-3 is an infectious agent with no clear evidence of disease causality, so, more studies are needed to unequivocally associate clinical pictures with its infection. So far, its association with reproductive disease seems to be the stronger link.

Finally, PCV-4 is a very recently discovered porcine circovirus and, so far, minimal information is available from it. It is not still known if participates or not with any disease causality. Moreover, its distribution all over the world is also unknown.

Glaesserella australis: another pathogen that I should be concerned about?

By Conny Turni
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The family *Pasteurellaceae* contains a range of haemophilic bacteria which colonise the upper respiratory tract of pigs. The most important ones are *Actinobacillus pleuropneumoniae* and *Glaesserella* (*Haemophilus*) *parasuis*. Some of the secondary haemophilic pathogens are *Actinobacillus indolicus*, *A. minor/porcitonsillarum* and *A. procinus*. To this list we now have to add a new species *Glaesserella australis*.

This new species was first recognised as being different when we looked at 37 isolates via multilocus sequencing analysis using three genes (*recN*, *rpoA* and *thdF*) according to the Kuhnert and Korczak (2006) protocol, and discovered that 17 isolates belonged to a new species.

Further isolates were gathered until we ended up with 29 isolates from 14 farms (free range farms as well as conventional farms). These isolates were analysed phenotypically and genotypically (Turni et al 2020).

Comparison of the 16S rRNA gene sequence similarity of *G. australis* and other members of the *Pasterellaceae* family revealed that *G. australis* was most similar to *Glaessserella* (*Haemophilus*) *parasuis* and *Actinobacillus indolicus*.

Phenotypic analysis revealed that *G. australis* is satellitic, not haemolytic, Gram negative, catalase negative, oxidase positive (weakly), indole positive and urease negative. The distinguishing features are catalase negative and indole positive reactions. *G. parasuis* and *A. indolicus* are catalase positive and *G. parasuis* is indole negative while *A. indolicus* is indole positive. Sugar fermentation also shows differences for *G. australis* with positive reactions for the fermentation of D-(+) Galactose, D-(+) Trehalose and D-(-) Arabinose (**Table 1**).

The isolates of *G. australis* came from small and large commercial farms, from free range and conventional farms, from indoor and outdoor farms. Some farm had other pathogens (*A. pleuropneumoniae, Pasteurella*

Table 1. Sugar fermentation patterns of haemophilic, non-haemolytic bacteria associated with the porcine respiratory tract

Species	D-(+) Galactose	D-(+) Trealose	D-(-) Arabinose
A. *indolicus*	-	-	+
G. *parasuis*	W+	-	-
G. *australis*	+	+	+

multocida, Truperella pyogenes, G. parasuis or *Streptococcus suis*). Other farms had no other respiratory pathogens.

Two scenarios of signs of disease were observed:

1) Most farms had no signs of respiratory disease on farm. However, at the abattoir higher pleurisy, lung lesions and lung abscesses were observed. One such farm with this scenario had good air quality with progeny raised in straw-based shelters and was free of *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae* and internal and external parasites. This farm upon identification of *G. australis* on their farm gave antibiotics specifically targeted at *G. australis*. This treatment gave a reduction in lung lesions from 40% to 23% prevalence, less lung abscesses (**Figure 1**) from 22.5% to 13.5%, less pleurisy from 13.8% to 11.5% and also no heart disease, which was previously at 2% associated with lung abscesses and pleurisy (all observations at the abattoir).

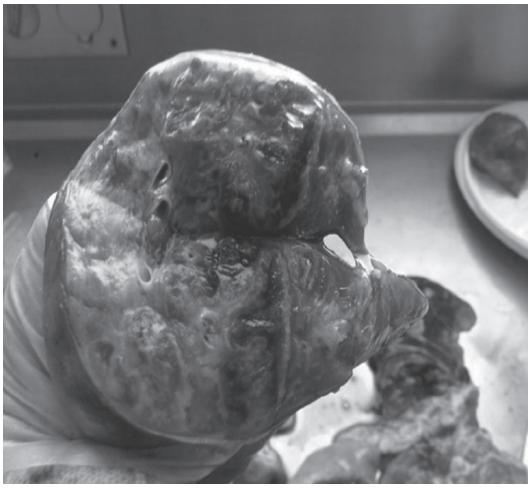

Figure 1. Chronic encapsulated abscess within the lung lobes without apparent necrosis or haemorrhage.

2) The other scenario was death on farm. Samples from dead pigs on two farms were sent to us for isolation and identification. One farm had dead pigs at 12, 16 and 20 weeks of age with lesions affecting up to 50% of the lung, no gross abscesses or pleurisy. The on-farm observations were dead pigs with purple extremities with multifocal necrotising and fibrinosuppurative bronchopneumonia, rapid necrosis or autolysis of affected lung area, which was 50% of lung with consolidated dorsal lung lesions (**Figure 2**). In one of these cases, we obtained a pure culture of *G. australis*.

The other farm gave no medication to the pigs and observed coughing, slower growth rate and increased death rate in the grower phase around 12 – 14 weeks (**Figure 3**).

In another case, we sampled 15 lungs and isolated *G. australis* from six of the lungs, which yielded pure *G. australis*. The observation overall is that many of the lesions resembled *A. pleuropneumoniae* lesions (**Figure 4**).

We have developed a multiplex PCR to identify the three main respiratory bacterial pathogens that affect pigs in the age bracket of 12 to 20 weeks, which are *Actinobacillus pleuropneumoniae*, *Pasteurella multocida* and *G. australis*.

To understand the impact of yet another pathogen we have to look at another study. Some years ago we did a study of pigs with pleurisy at the abattoir. We sampled lungs with pleurisy from 46 batches with each batch

Figure 2. Pigs found dead with purple extremities with lung lesions with autolysis or rapid necrotising lesions and fulminant pneumonia.

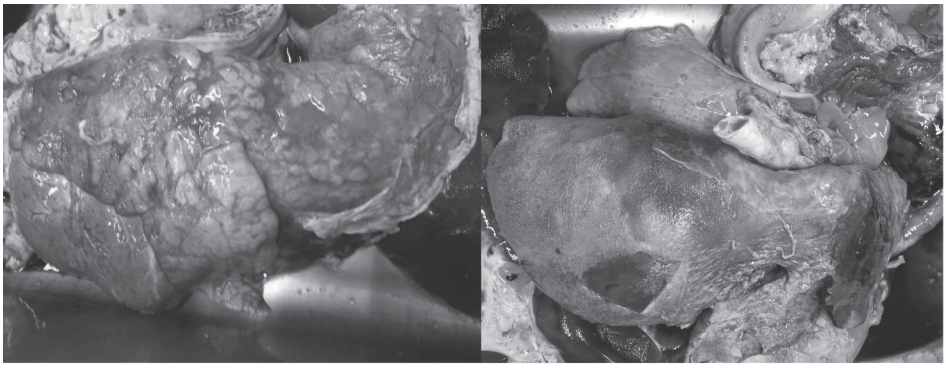

Figure 3. Lungs from 12 to 14 week old pigs dying on farm.

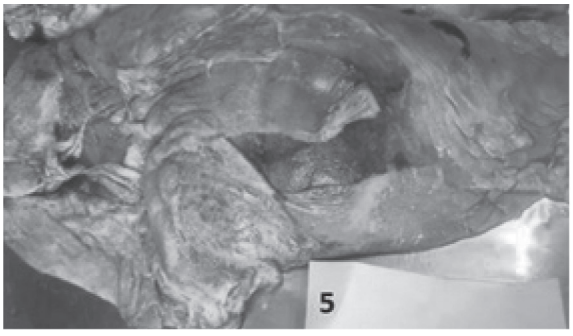

Figure 4. Lesion from which pure G. australis was cultured.

representing a farm. From each batch we sampled five lungs (if we could find 5 lungs with pleurisy) and investigated the respiratory bacterial pathogens and the only viral pathogen of consequences in Australia, which is PCV2

We found synergistic pathogens: 34 farms *Mycoplasma hyopneumoniae* and 31 farms had PCV2. There were also other primary pathogens: *A. pleuropneumoniae* from seven farms and *Glaesserella parasuis* from one farm.

The abundance of secondary pathogens was astounding with 38

farms with *Streptococcus suis*, 29 farms with *Actinobacillus* species (not *A. pleuropneumoniae)*, 24 farms with *P. multocida* (even though *P. multocida* can also be a primary pathogen (de Oliveira et al 2018), *M. flocculare* on nine farms and *M. hyorhinis* on four farms and one farm with *S. porcinus* and one farm with *S. minor*.

For six pathogens there was evidence of an association between more pathogens present and pleurisy category (>10% pleurisy). We did not find a clear link between certain pathogens and high pleurisy. The high percentage of farms with synergistic pathogens is important, combined with the finding of high prevalence of secondary pathogens.

Studies have shown that PCV2 can reduce acquired immunity to other pathogens and therefore affecting co-infection with secondary pathogens and pleurisy and lung lesions (Opriessnig et al 2011; Wellenberg et al 2010). Both synergistic pathogens identified in our study, *M. hyopneumoniae* and PCV2, have been established to increase severity of respiratory disease (Opriessnig et al 2011).

Not all farms with high pleurisy had a combination of pathogens, which points to other contributing factors such as: parasites and non-infectious factors such as environment, management, use of antibiotics and pig factors.

Studies have shown that parasites can affect the immune response to vaccination against and challenge with *M. hyopneumoniae* (Steenhard et al 2009).

Air pollution due to ammonia and particles having a negative effect on the respiratory system and hence less resistance to respiratory problems has been well documents (Banhazi 2013).

The biggest risk factors are systems that are not based on all-in all-out systems. Systems where pigs with an age difference of more than one month are reared in the same air space and where pigs are repeatedly mixed are recognised as problematic (Eze et al 2015; Merialdi et al 2011).

So even though we just discovered a new pathogen, I personally do not think things have changed, we have just become better at isolating pathogens and at identifying them as new pathogens. We suspect there are more new pathogens as our preliminary research indicates.

Summary

I think we need to focus on:

- Controlling the pathogens that cause synergy
- Controlling other factors via management
- Enhancing the immune system and strive towards a healthy respiratory microbiome
- Rethink the use of antibiotics.

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How to minimize the impact of swine influenza in the farm

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Introduction

Influenza is one of the top viral diseases that producers and veterinarians have to deal with on a regular basis. Dealing with influenza has become increasingly difficult since the emergence of the 2009 H1N1 pandemic virus, the on-going incursions of human seasonal viruses and the establishment of swine endemic viruses with genes of human and avian origin in the pigs. This change in influenza landscape started in the late 90's and has been accentuated and became worst during the last decade. Thus control of influenza using traditional approaches has become frustrating and has challenged the industry to seek newer ways to control influenza infections.

There have been production systems that have made control of influenza a priority due to the economic losses influenza represents for them and there have been attempts to eliminate the influenza virus from selected breeding stock herds. All these efforts have contributed to further understand options for influenza control in the field. Our group has focused on the study of influenza transmission within farms with the main goal to provide recommendations to prevent, control and eliminate influenza in pigs. In this paper we will summarize the most relevant findings, some opinions, and lessons learned that hopefully will help us point towards actions to control influenza in pigs more effectively.

Consider having an influenza negative pig at weaning

Having herds influenza negative would be desirable, if we could maintain them as such. There are key inputs to farms that are required to be influenza negative consistently if the herd is to remain negative in the long run. The consistent introduction of negative gilts is one of the most important factors to prevent introduction of new influenza infections. Introduction of positive gilts has been associated with weaning influenza positive pigs (Chamba et al., 2018).

Currently the majority of gilt protocols do not prioritize the prevention

of influenza infections. Unless these protocols change, and often that may require adding segregated rooms/barns, it may be easier to focus our efforts on developing vaccination strategies and protocols to contain infections within the gilt development unit (GDU). These protocols should include limiting the movement of people between the GDU and the rest of the farm, preventing movement of contaminated fomites between the GDU and the different farm areas and transferring non-shedding gilts from the GDU to the breeding area. In general there is a lack of understanding on what to do in gilts to minimize the impact of influenza infections to the rest of the breeding herd.

The role of people at introducing influenza infections into pig herds is another factor that feels like a wild card for now. Genetic analyses of virus sequences conducted over the last decade have emphasized that people can play an important role at introducing influenza viruses into pigs. It is not uncommon to report influenza like sickness in farm employees that then it is followed up with similar sickness in the pigs and the subsequent detection of influenza strains of human origin in the pigs. Thus the role of people cannot be underestimated but we still lack a clear understanding on how often humans introduce new viruses into pigs and what we can do to prevent these introductions from happening. There are spill over events of human origin influenza viruses into pigs. These viruses then can reassort with pig viruses, become endemic in the pigs, and then the pigs disseminate the viruses between farms.

Lastly, after elimination, leaving herds completely naïve may not be desirable. Given the ubiquity of influenza viruses, naïve herds would be acting as magnets for viruses that if infected, they would amplify and disseminate infections of major impact. A better outcome under this scenario would be to have herds that are immune but have no virus circulating, or at least circulating at a very low level.

Key points towards weaning influenza negative pigs

1. Minimize the introduction of viruses via gilts and keep the influenza viruses in the GDU

Gilts have been associated with the introduction of IAV in several studies. Groups of gilts within the first 30 days post delivery into the isolation/GDU were more likely to test positive than groups that had been already in the GDU for more than 30 days (Diaz et al., 2015). This indicates that gilts either served as a source of new viruses or that they were able to amplify flu infections post arrival. In another study, introduction of positive gilts was associated with detection of influenza virus in pigs at weaning (Chamba et al.,2018).

Thus protocols for introducing gilts need to be adapted to include influenza measures.

One measure that can be done to decrease the shedding of IAV within the GDU is vaccination. Vaccines are helpful at improving the clinical signs due to IAV infection and can also help decrease shedding and transmission within a group (Romagosa et al., 2011). However, picking up the right vaccine may be a challenge but historical information on the strains circulating in the gilt source herd, in the GDU itself and the region should help educate that decision.

The other source of influenza spread to the rest of the farm is via contaminated fomites and hands/skin of farm workers interacting with the gilts that then become in contact with other pigs in the farm. Special attention should be placed at having dedicated materials to the GDU and at limiting the movements between the GDU and the rest of the farm. Influenza is a very contagious virus and transmission via fomite materials and personnel working with animals has been shown experimentally (Allerson et al., 2013).

2. Protect the piglet from birth to weaning

Suckling piglets play a very important role at maintaining influenza infections endemic in the breeding herds and at disseminating the virus to other farms at weaning. Piglets are born IAV negative; however, in endemically infected breeding herds, it is common for piglets to become infected before weaning. About 28% of weaned groups out of 1,523 tested positive for IAV at weaning (Chamba et al., 2017). Furthermore, co-circulation of distinct influenza viruses is common in piglets which results in co-circulation of strains in nurseries and finishers. Understanding how piglets become infected during the suckling period and preventing them from getting infected in the first place should help wean an influenza negative pig.

In endemically infected breeding herds, piglets basically have 3 weeks to become infected since most common weaning age ranges from 18 to 24 days. It is common to detect ramping levels of IAV infection in the second week of age resulting in highest prevalence around weaning age. However, depending on the farm, there is the possibility that the pigs become exposed during the first few days of age likely as a result of moving pigs between litters or exposing them to contaminated fomites. In high prevalence farms it is not uncommon to find IAV contaminated crates, aerosols, equipment and materials.

There are management practices during the suckling period that may facilitate the spread of IAV among and between litters. Among these practices, the use of nurse sows has been demonstrated to contribute to the dissemination of IAV in a controlled study (Garrido et al., 2020). Nurse sows are dams that adopt younger pigs from different litters in an attempt to improve piglet survivability and increase the number of pigs weaned.

Most commercial farms commonly select nurse sows that have good milk production and have recently weaned their own pigs. In a recent study by Garrido et al., (manuscript under preparation), we evaluated IAV status in litters of pigs adopted by nurse sows and compared it to the IAV status of litters reared by the piglets' biological mothers. A number of nurse sows had viable virus at the time of adopting the new litter which suggests these nurse sows serve as a possible source of IAV infection to the newly adopted piglets. Litters of piglets adopted by the nurse sows were more likely to test IAV positive and became infected with IAV more rapidly than litters of piglets from control sows. However, the impact of using nurse sows on IAV prevalence became less significant as piglets became older and at weaning there were no differences in IAV status between litters of nurse and control sows. In addition, there was also evidence of sows becoming infected during the lactation period. Overall, these results provide strong evidence that nurse sows play an important role in transmitting IAV to piglets and maintaining IAV infections endemic in breeding herds.

Cross-fostering and in particular movement of pigs between rooms also facilitates the spread of virus during the pre-weaning period. Special attention should be placed at limiting movement of pigs between litters/rooms if we seek to wean an influenza negative pig.

In addition to limiting exposure to influenza infections, increasing the resistance of the piglet to becoming infected during the preweaning period should be considered and it can be achieved by having an immune pig. The most common way to do so is by using sow vaccination either pre-farrowing or mass vaccination. Sow vaccination has as a goal to increase the transfer of maternal (passive) immunity to piglets through colostrum. Maternal immunity can protect piglets from clinical signs despite being infected ("silent carriers") and can decrease transmission of IAV if immunity generated by the vaccine can neutralize virus infections. The impact of passive immunity on influenza transmission differs slightly from the impact of active immunity. In the case of pigs that received passive immunity generated by vaccinating dams with an autogenous vaccine that perfectly matched the challenge virus, R (reproduction ratio which is a measure of virus spread within a population) was estimated at 0.84 (CI 0.05-3.68) (Allerson et al., 2013). The confidence interval obtained for this R value indicates that although in many cases passive immunity may result in limited transmission, in other cases it illustrates that spread of IAV within populations can still take place. In contrast, the R value for pigs with heterologous passive immunity was estimated at 7.81 (CI 4.57-12.56) which indicated that IAV could transmit efficiently despite the presence of immunity. Therefore, although maternal immunity can be protective, immunity levels wane as piglets get older and there is variability among the pigs within a group. Although it helps to have an immune piglet, immunity by itself is not enough to result in the weaning of a pig that is consistently

influenza negative.

Furthermore, the impact of sow vaccination on IAV infection of piglets at weaning has been evaluated epidemiologically. Both, prefarrow and mass sow vaccination protocols have been shown to decrease the number of infected groups of pigs at weaning, and the prevalence within those groups. Interestingly, in this study both types of vaccines, commercial and autogenous had similar results in a study by Chamba et al (Chamba et al., 2020) suggesting that sow vaccination can be used to lower the level of infection at weaning. In a different study, use of sow vaccination was one of the few parameters also associated with lower levels of IAV infection at weaning (Chamba et al., 2017). Thus the combination of sow vaccination to improve the resistance of the pig to IAV infections and the implementation of measures to limit IAV exposure to suckling pigs seems the best approach to improve the chances to wean an influenza negative pig.

3. Reinforce general biosecurity practices

Weaning an influenza negative pig is only sustainable if we can keep external viruses out of the farms. In the case of influenza, it is very important to strengthen external biosecurity practices in particular to limit the introduction of contaminated materials and mitigate infections transmitted via people. In terms of people, the added requirements should limit personnel with influenza like illness to report to work. Use of N-95 masks and disposable gloves are also recommended to mitigate the bi-directional transmission of IAV. Lastly, seasonal vaccination of workers is highly recommended although we do not know the real impact it has on preventing IAV transmission from people to pigs.

Lastly, internal biosecurity measures that limit movement of personnel between contaminated and non-contaminated areas within a farm, and limiting movement of equipment and materials between farrowing rooms should be evaluated and implemented when possible.

Summary

A key aspect of influenza control appears to be to wean an influenza negative pig. Although some questions remain unanswered, significant advances have been made to understand influenza transmission within farms and factors that help maintain infections endemic. Focusing on the suckling piglet as the goal for influenza control should help production systems prioritize the actions to take at the company level to control influenza. Actions such as introducing negative gilts (or at least isolating them and wait to recover from infection before entering them into the herd), use of vaccination to minimize

IAV transmission and clinical impact, implementation of management strategies to keep piglets free from exposure and having procedures in place to limit introduction of infected farm personnel should be at the core of controlling influenza in pigs. Having an influenza negative pig at weaning should be desirable not only to limit production losses but also to limit the risk of IAV transmission to people.

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Glaesserella parasuis: how to keep this pathogen under control in farms?

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1. Introduction

Glaesserella parasuis is a Gram-negative bacterium that exclusively infects pigs. This organism is considered an early colonizing agent that, under appropriate conditions, induces a severe systemic inflammatory pathology, called Glässer's Disease (GD), which is considered to be one of the principal bacterial disorders emerging in the pig production.

GD occupies a prominent position among the main infectious challenges in nursery phase. Many causes can explain the increase in clinical cases of GD and, among them, the following stand out: management (mixing pigs with different microbiological and immunological backgrounds); the use of vaccines with limited or even no cross-protection potential; the circulation of strains of *G. parasuis* that are highly virulent and capable of triggering Glässer's Disease in healthy conventional animals (primary pathogen profile); viral (Porcine Circovirus and Influenza A Virus) and bacterial co-infections (*Mycoplasma hyopneumoniae*, *Bordetella bronchispetica*, *Pasteurella multocida*, and *Actinobacillus pleuropneumoniae*), which, in general, facilitate the infection process by *G. parasuis*.

Economically, uncontrolled infections produced by *G. parasuis* result in highly significant losses, which can exceed 80 million dollars per year for the pig production chain (Holtkamp et al., 2006). Losses are the sum of several variables, such as a: growth delay; b) increase in the feed conversion index; c) decrease in daily weight gain; d) high costs derived from the use of antibiotics; e) veterinary technical assistance; and f) increased mortality rates that can reach 10% (Oliveira et al. 2004).

GD control represents one of the main challenges for clinical veterinarians and the veterinary pharmaceutical industry, mainly due to the antigenic characteristics of *G. parasuis*. Clinically, the microbiological characterization of GD outbreaks is essential for effective treatment and prevention of future

outbreaks. For the pig industry, research targeting protective structural antigens is the way to obtain modern vaccines capable of providing heterologous protection against this antigenically complex pathogen.

In this review, we will address the main mechanisms used by *G. parasuis* to produce GD. In addition, we will present and discuss rational strategies that should be used to characterize clinical outbreaks of GD, as well as to institute procedures capable of controlling infections produced by the agent at the farm level.

2. *Glaesserella parasuis* and its phenotypic diversity

G. parasuis is a very complex microorganism from a phenotypic and pathogenic point of view and, currently, the classification of all known serovars (SVs) are according to virulence and capsular type into three distinct groups: SVs 1, 5, 10, 12, and 14 make up the group of SVs considered to be highly virulent; SVs 2, 4, 8 and 15 constitute the group considered to be of moderate virulence and SVs 3, 6, 7 and 9 are classified in the group of low virulence or even avirulent (Kielstein & Rapp-Gabrielson 1992). The initial studies regarding the classification of *G. parasuis* virulence in terms of capsular type are very consistent for most SVs. However, recently, we demonstrated that the reference strain for SV7 (strain 174) was highly virulent in pigs (Guizzo et al. 2018). In addition to all the lesions described during severe episodes of GD, it was also capable of inducing two new lesions: endophthalmitis and thymic lymphoid depletion (Dazzi et al. 2020).

Infections caused by *G. parasuis* have already been described in all countries with intensive pig farming and, in Brazil, clinical cases of GD have been observed with increasing frequency and triggered by a very diverse

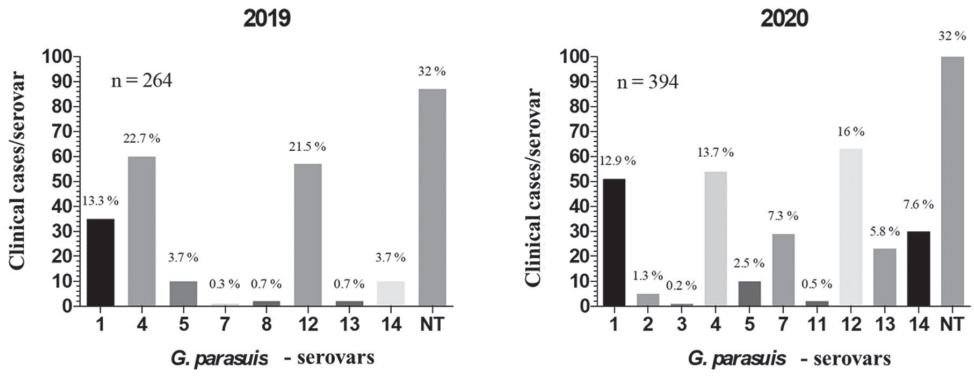

Figure 1. Distribution of Glaesserella parasuis serovars associated with clinical cases of Glässer disease in Brazil. A total of 658 clinical strains of G. parasuis were included in this analysis; of these, 264 strains were isolated in 2019 and 394 isolated in the first half of 2020.

panel of serovars of *G. parasuis*. Recently, through a robust typification study involving 459 Brazilian clinical strains of *G. parasuis* isolated over 20 years (1987 - 2016), we demonstrated the circulation in our herds of 9 different serovars (SV1, SV2, SV4, SV5, SV7, SV12, SV13, SV14, and SV15). In addition to that, an expressive number of nontypeable strains (NT), which were classified molecularly into nine different profiles (Pires Espindola et al. 2019; Prigol 2019). Also, as illustrated in **Figure 1**, our current results demonstrate that in 2019, and even in 2020, serovars NT, SV12, SV4, and SV1 have been the most frequently found in clinical cases of GD (AFK Imunotech, unpublished data). Also, we highlight that in 2019 we identified two occurrences of GD caused by SV8 and, in 2020, other clinical cases caused by SV3 (n = 1) and SV11 (n = 2).

3. When and how do piglets become infected with *Glaesserella parasuis*?

Piglets are commonly colonized in the first week of life by strains of *G. parasuis* that are present in pre-weaning; therefore, the sows are the main reservoir and transmitters of *G. parasuis* to the piglets. In general, during the pre-weaning phase, maternal antibodies transferred through colostrum control the evolution of the infection. The positive effect of colostrum on the reduction of colonization by *G. parasuis* on piglet's respiratory mucosa has already been demonstrated (Cerda- Cuellar et al. 2010). Therefore, the vaccination of sows is a considerable strategy to reduce the bacterial load of *G. parasuis* that can reach the respiratory mucous membranes of piglets during pre-weaning and, consequently, the agent transmission in the nursery phase.

Many piglets have their first contact with *G. parasuis* in the nursery when animals from different origins are mixed. In this case, transmission occurs mainly through direct contact between piglets free of *G. parasuis* and those colonized by virulent strains, but which did not manifest clinical disease due to the presence of maternal immunity or active immunity. Still, in more complex scenarios, when mixing pigs infected by different SVs, it is possible to observe clinical cases of GD produced simultaneously by diverse virulent SVs. The contact with *G. parasuis* presents in the environment and the airborne transmission (it is believed that the agent can be transported over short distances by air) must be considered as the primary form of infection. Without a doubt, mixing piglets from different origins is the main trigger for the development of GD in the current context.

When infection occurs for the first time on a farm, it is possible to observe super-acute clinical presentations, with sudden deaths, after an incubation period of 7 to 10 days. On the other hand, in farms with reinfections, animals usually develop the classical picture of the disease, and in farms with endemic infection, only piglets from negative origins develop the disease. Still, although the disease manifests itself mainly in the post-weaning phase, pigs of any age are susceptible when a virulent and antigenically different strain is introduced on the farm (Oliveira & Pijoan 2002).

4. How does *G. parasuis* produce Glässer disease?

Once inside the upper respiratory tract, *G. parasuis* secretes a protease that specifically breaks down mucosal IgAs (Mullins et al. 2011) and allows itself to migrate efficiently to the maxillary sinuses (Frandoloso et al. 2020, in preparation). The infection can progress to the middle ear and, at the same time, to the trachea, where virulent strains of *G. parasuis* adhere with great avidity to the epithelial cells (Vahle et al. 1997).

In the lung, *G. parasuis* finds a hostile immune environment, and its survival is conditioned by its ability to evade responses from pulmonary sentinel cells, especially that of macrophages. Virulent strains of *G. parasuis* can delay the phagocytosis process through two surface proteins called VtaA8 and VtaA9 (Costa-Hurtado et al. 2012) and decrease the synthesis and surface expression of SLA-II molecules (Frandoloso et al. 2012). Through these mechanisms, the bacterium remains viable for a longer time in the pulmonary environment, slows down the development of specific immunity, and manages to achieve its great goal, entering the systemic blood circulation and migrating to the serous.

Currently, the available data on the pathogenesis of *G. parasuis* do not allow us to clearly understand all the steps of the infection produced by this agent. In this context, we demonstrated that pigs challenged with a virulent strain (Nagasaki, SV5) by the intratracheal route develop bacteremia within 12 hours of the challenge, suggesting that the pulmonary route is very efficient in facilitating the pathogen's access to blood circulation (Frandoloso et al 2011). On the other hand, we demonstrated that pigs challenged by the intranasal route with different strains of *G. parasuis* (SV1, SV5, SV7, and NT) consistently develop, after 36 hours of the challenge, an intense systemic inflammatory response, however, without pulmonary lesions (pneumonia). These data allow us to highlight that, during the natural infection process, *G. parasuis* can reach the blood circulation directly from the upper respiratory tract and cause GD.

Once in the circulatory system, the development of GD will depend on the ability of *G. parasuis* to overcome the attack of the innate immune system. Some years ago, we demonstrated that, during the systemic phase of the infection, *G. parasuis* induces depletion of the principal subpopulation of T lymphocytes that circulate in the peripheral blood of swine, consisting of TCR$\gamma\delta$ lymphocytes (Frandoloso et al. 2012). These lymphocytes are the only ones that can act directly on bacteria and viruses regardless of the SLA-I implication, being, therefore, a strategic target for *G. parasuis*. The mechanism by which the agent kills TCR$\gamma\delta$ lymphocytes is under investigation in our laboratory, and in this particular case, we have demonstrated that the bacteria produces a depletion of thymic lymphocytes, and this may be one of the causes to explain the decrease in these peripheral blood lymphocytes. (Dazzi et al. 2020).

In addition, *G. parasuis* alters (decreases) the surface expression of SLA-II

molecules in monocytes, compromising the functional ability of these cells (Frandoloso et al. 2012). Regarding neutrophils, the phagocytosis of *G. parasuis* is only efficient when the bacteria is opsonized by antibodies (IgGs) (Barasuol et al. 2017), suggesting that *G. parasuis* has mechanisms that hinder the normal phagocytosis process by neutrophils, which play a crucial role against blood transition bacteria.

Still in the blood, *G. parasuis* needs to resist the attack of the complement system. In this respect, Wang et al. (2018) demonstrated that the sialylation of the lipo-oligosaccharide (incorporation of N-acetylneuraminic acid into the galactose terminal residue) gives the virulent strains (lsgB + gene) the ability to resist to the attack of the alternative pathway of the complement system, which is an essential condition for the bacteria can reach the host's different serosa.

Throughout this complex process, *G. parasuis* needs to acquire iron from the host to stay alive (necessary for energy generation, DNA replication, oxygen transport, and protection against oxidative stress) and the advance of the infectious process. In pigs, iron is almost entirely associated with intracellular (ferritin, hemoglobin) or plasma (transferrin) proteins, a fact that restricts the access of this molecule to bacteria, a phenomenon now known as "immunological nutrition". *G. parasuis*, as well as *Actinobacillus pleuropneumoniae*, has a sophisticated surface protein system consisting of the TbpA and TbpB proteins (transferrin A and B binding proteins) capable of removing iron from swine transferrin. These proteins, besides vital for the survival of *G. parasuis*, constitute excellent vaccine antigens (Frandoloso et al. 2015).

Once *G. parasuis* overcome all confrontations with the components of the immune system, it starts its replication at specific sites, such as the synovial membrane, peritoneum, pericardium, pleura, and meninges. The bacterium's access to these serous cells is mediated by its ability to adhere to and invade endothelial cells (Frandoloso et al. 2013b). Finally, in the target tissues, the pathogen triggers an intense inflammatory response.

5. Clinical and pathological presentation of Glässer's Disease

It is possible to observe four clinical forms during infections produced by *G. parasuis*: Glässer Disease (fibrinous polyserositis), septicemia (without polyserositis), acute myositis (in the masseter muscles), and the respiratory form (bronchopneumonia). Different studies demonstrate that different strains of *G. parasuis*, serovars, and concentrations can cause GD. (Blanco et al. 2004; Dazzi et al. 2020; Frandoloso et al. 2011; Guizzo et al. 2018; Oliveira et al. 2003). The immunological status of the pigs and the way they are obtained (conventional, specific-pathogen-free - SPF, and deprived of colostrum) must be taken into account when designing experiments related to the pathogenicity of strains or even vaccine studies. Our experience in this area allows us to state that the animal model significantly impacts the

controlled clinical development of the disease.

Fibrinous polyserositis. The course of the disease is acute and mainly affects animals aged 5 to 12 weeks. Clinically, high fever (> 40.5°C) is observed, followed by a lack of appetite and apathy. On some occasions, due to the high transcription of TNF-α (Frandoloso et al. 2013a), it is possible to observe cyanotic areas in the skin (peripheral circulatory failure). The animals' breathing is usually affected (increased and with an abdominal appearance), as well as the heart rate (tachycardia). Joint problems are quite common (arthritis, especially in the radiohumeral joint) (Dazzi et al. 2020), and in some epidemic outbreaks, it is possible to observe neurological signs compatible with meningitis.

The lesions that characterize this form of the disease are polyserositis and fibrin-purulent polyarthritis. It is quite common to observe the deposit of large amounts of fibrin on the abdominal and thoracic organs. Abundant serofibrinous fluid can be seen in all cavities and also in the pericardial sac. On the other hand, in the joints, an increase in less viscous synovial fluid is observed. Lesions in the central nervous system are characterized by the opacity of the meninges, especially those covering the cerebellum (a strategic area to isolate *G. parasuis*) (Dazzi 2018).

Septicemia. In cases of septicemia, the animals are apathetic, depressed, dyspnoic, cyanotic, and present hyperthermia (~ 41°C). Changes in blood clotting decreased platelet count, and leukopenia are seen 24 hours after infection. At necropsy, hemorrhage foci are observed with petechiae in some organs (Amano et al. 1997). Histopathology shows the presence of fibrin microthrombi in the lungs, brain, and kidneys. The bacteria can be seen inside small vessels and in the cytoplasm of phagocytes that form the inflammatory infiltrate (Amano et al. 1997; Martin de la Fuente et al. 2009).

Masseter muscle myositis. Hoefling (1991) described this form of the disease after infecting specific-pathogen-free gilts (SPF) with *G. parasuis*. The animals presented hyperthermia, lack of appetite, weakness, and ataxia; however, the most significant feature was observed in the head, which appeared swollen, with large cyanotic areas. During the histopathological study, it was observed suppurative submandibular lymphadenitis and the presence of serofibrinous exudate containing an abundant number of inflammatory cells in the subcutaneous tissue that extended through the perimysium and endomysium of the masseter muscle.

Respiratory form. Respiratory conditions characterized by cough and dyspnea can be observed. Sneezing is frequent after the intranasal challenge with *G. parasuis*. Catarrhal-purulent bronchopneumonia and, in some more severe cases, fibrinorrhagic bronchopneumonia can be seen after the experimental challenge with *G. parasuis* (Rapp-Gabrielson et al. 2006; Rapp-Gabrielson 1999).

6. Diagnostic strategy

The diagnosis of GD can be made by observing the clinical history, symptoms, and injuries of the animals. Since many of the symptoms are common to other swine infections, it is essential to use microbiological, molecular, and immunological tools to identify the presence of *G. parasuis* in the affected animals.

Anatomopathological study. The post-mortem examination is the first guiding approach to GD diagnosis. The macroscopic lesions observed are characterized by the presence of serous or fibrin-purulent exudate on the serous surface, usually in the peritoneum, pleura, pericardium, joints, and meninges. In our studies, we observed that animals submitted to experimental infections often develop bronchopneumonia with local or multifocal cranio-ventral consolidation, or even interstitial pneumonia (Dazzi 2018; Frandoloso et al. 2011).

Although macroscopic lesions associated with clinical signs are, in most cases, quite convincing about a possible episode of GD, isolation of the agent remains essential to institute correct treatment with antibiotics and to design an assertive preventive program. In this sense, we represent in **Figure 1**, based on our experience, the list of samples that must be collected during a necropsy to be sent to the bacteriological diagnosis laboratory. It is important to remember that *G. parasuis* is a microorganism that primarily produces a systemic inflammatory disease, not pneumonia.

Therefore, the isolation of *G. parasuis* from systemic sites is essential to define the strain (s) that are causing the clinical case (Frandoloso 2019).

This phase is especially important since the success of *G. parasuis* isolation is conditioned to the quality of the sample collection procedure (the samples collection must be aseptic). [1]During a clinical case, we recommend that at least five animals are selected per production unit to perform necropsy and material collection. This number is necessary because more than one serovar of *G. parasuis* may be circulating on the farm and causing GD. [2]All swabs that will be sent for bacterial isolation must contain Stuart's or Amies transport medium. [3]The cerebellum is frequently affected during the systemic phase of the infection, and, therefore, we recommend that the animal's head be sent to the laboratory to proceed with the collection of material in aseptic conditions, avoiding contamination by other fast-growing organisms. [4]Cases of pericarditis are frequent in GD. For the isolation of the growing agent, free of any other microorganism (pure), we recommend sending the organ together with the lungs and with the intact pericardial sac. [5]Joint fluid can be collected using a 21Gx14" needle. The needle insertion site must be disinfected (70% alcohol or 2% chlororedixin) or cauterized. It is necessary, after collection, to keep the needle attached to the syringe and that the plunger remains retracted. If it is not possible to aspirate joint fluid, send the closed joint to the

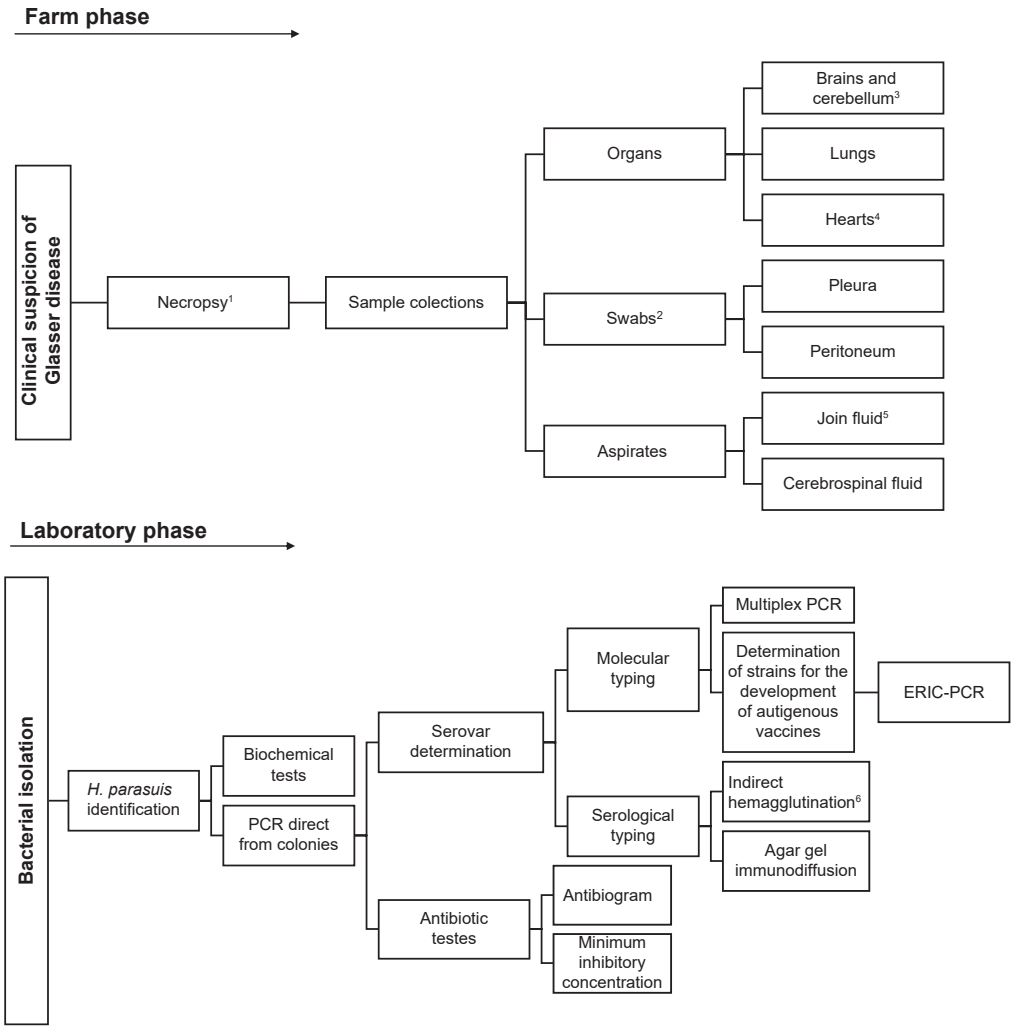

Figure 2. Strategic flowchart for collecting and processing clinical samples from animals suspected of suffering from Glässer disease. Farm phase

laboratory. **Laboratory phase.** In this stage, the microorganism is isolated and characterized.[6] *G. parasuis* can be typed using multiplex PCR (currently used in our laboratory) or through serological tests such as indirect hemagglutination (IHA) and Agar Gel Immunodiffusion (AGID). The IHA technique is more specific than the AGID; however, both are less specific and discriminatory than multiplex PCR (Frandoloso 2019).

Also, in the histopathological study, it is possible to observe fibrinopurulent inflammation, with infiltrates of neutrophils, macrophages, and other inflammatory cells in affected organs. Vascular disorders are frequently seen in cases of septicemia, as well as edema, hemorrhages, and thrombi in the brain (in severe cases), lungs, liver, spleen, and kidneys. The formation of

thrombi and microthrombi is associated with the endotoxins released by the bacteria during infection. The pathological result consists in the development of a condition compatible with disseminated intravascular coagulation (DIC) (Amano et al. 1997).

Direct bacteriological diagnosis. This diagnosis consists of the confirmatory procedure for Glässer's disease. The isolation of the agent is carried out from the samples described in figure 1, and success depends on two considerable factors: a) the collection procedure performed by the veterinarian; and b) the sample transport time to the laboratory. Regarding the first, we advise that the necropsy be conducted first with the aim of collecting samples for the microbiological study, that is, avoiding to the maximum the exaggerated opening of the cavities during the collection of swabs and, always, using sterile or disinfected necropsy tools. Subsequently, an investigation of macroscopic lesions and tissue collection can be conducted. Regarding the transport time, it is indispensable to pack samples in thermal boxes with an internal temperature of 4 - 8ºC and that they arrive at the laboratory within 24 - 36 hours after collection. The success of *G. parasuis* recovery after 48 hours is considerably low, mainly due to tissue proteolysis (pH below 6.2 induces the death of *G. parasuis*).

In the laboratory, the samples are seeded in media suitable for the growth of *G. parasuis*. In this particular case, the chocolate agar supplemented with NAD, glucose, and IsoVitaleX ™ provides more nutrients to the microorganism compared to any other culture medium. The isolation of *G. parasuis* is often complicated by contamination by other fast-growing bacteria (principally when sample collection is not performed correctly); therefore, the use of bacitracin in the culture media can facilitate the recovery of this microorganism in pure cultures (Miani et al. 2017).

Although the identification of *G. parasuis* colonies can be carried out through biochemical tests, currently, several molecular identification options utilizing PCR are available to accelerate the agent identification process (Angen et al. 2007; Oliveira et al. 2001; Turni et al. 2010). In GD, it is essential to recover systemic strains and, even more, to define the serovar of strains recovered from all systemic sites and all animals with positive isolation. We often isolate more than one serovar of *G. parasuis* per farm (different serovars isolated from distinct animals), and we have also identified animals co-infected with two virulent serovars of *G. parasuis* (SV1 isolated from brain and SV12 isolated from peritoneum).

The typification process of *G. parasuis* has evolved tremendously in recent years. In the early 1990s, Kielstein & Rapp-Gabrielson (1992) used the Agar Gel Immunodiffusion (AGID) technique to define 15 reference serovars for this pathogen. Years later, Del Rio et al. (2003) presented Indirect Hemagglutination (IHA) as an alternative typification methodology for *G. parasuis*, with the main advantage over the AGID, better specificity, and fewer cross-reactions

between serovars. Recently, our group proposed a modification in the IHA (mIHA) technique, increasing the potential for resolution of the diagnosis and, fundamentally, the constancy and linearity of this technique (Lorenson et al. 2017).

Today, although AGID, IHA, and mIHA can be used for *G. parasuis*, molecular typing, through the multiplex PCR described by Howell et al. (2015) and Jia et al. (2017), has become popular. Rationally, the use of the technic makes more sense because of its easy execution, the discriminatory potential of serovars, and reproducibility among laboratories. All typification techniques are based on phenotypic (serotyping) or genotypic (multiplex PCR) characteristics of the 15 reference strains of *G. parasuis*. For this reason, a large number of clinical strains isolated from cases of GD that do not meet a pattern similar to the KRG method are classified as untyped strains.

In this regard, we recently presented a molecular strategy to differentiate untyped strains and demonstrated that the phenotypic diversity of *G. parasuis* is even greater than previously thought. At least nine different untyped strains circulate in Brazil (Espíndola et al. 2019), which allows us to understand better the enormous challenge of preventing GD through the use of usual vaccines (bacterins). It is worth mentioning that it is possible to use numerous molecular techniques for the typification of *G. parasuis* (de la Puente Redondo et al. 2003; Mullins et al. 2013; Turni et al. 2018). However, in our opinion and experience, PCR multiplex (Howell et al. 2015; Jia et al. 2017) is the most recommended.

Also, two molecular strategies are available to study the virulence of strains involved in a clinical case of GD (Galofre-Mila et al. 2017; Howell et al. 2017). The investigation of virulence and the study of the genetic diversity of clinical strains (Rafiee et al. 2000) are essential to define, with scientific criteria, the antigenic basis of an autogenous vaccine, when necessary.

Serological diagnosis. It is possible to detect the presence and circulation of virulent strains of *G. parasuis* in farms through serological tests. In this regard, it is worth noting that non-virulent strains that colonize the upper respiratory tract do not always induce immune responses with systemic repercussions, and this characteristic needs to be taken into account when certifying farms as negatives for *G. parasuis*.

Tests available for the evaluation of anti-*G. parasuis* antibodies (IgM and IgG) include the fixation of the complement system (FCS) (Takahashi et al. 2001) and the ELISA technique (Miniats et al. 1991; Segalés 1996; Solano-Aguilar et al. 1999). The ELISA technique has numerous advantages over FCS, among which we highlight mainly the specificity and reproducibility, being, therefore, the most indicated technique to evaluate the antibody response in pigs during infection processes (clinical and subclinical) and immunization.

Through an experimental approach, Macedo et al. (2016), demonstrated that the OppA protein (oligopeptide permease A) of *G. parasuis*, besides

immunogenic in swine, is an excellent antigen for the specific serological diagnosis of this agent. These authors presented the development of a specific Indirect ELISA based on this protein, and, currently, the test can be purchased commercially through the company BioCheck (*Haemophilus parasuis* Antibody Test Kit).

In parallel to the ELISA described by these authors, our group developed an ELISA capable of detecting and differentiating animals infected with virulent strains of *G. parasuis* from those colonized by strains unable to cause Glässer's Disease. This ELISA is based on the *G. parasuis* periplasmic iron-binding protein (FbpA) (Giacobbo et al. 2019).

Finally, the use of customized ELISAs based on strains of *G. parasuis* circulating on the farm is a relevant strategy to define vaccination protocols correctly in piglets. In this sense, we highlight that the quantification of maternal antibodies circulating in the piglet (quantitative ELISA) is the strategy to be followed and that frequent mistakes are made when making decisions based only on serum absorbance (qualitative ELISA).

7. Prevention of infections caused by *Glaesserella parasuis*

The prevention of Glässer's disease has been carried out for a long time, through the use of inactivated vaccines and formulated with one or two serovars of *G. parasuis*. Today, most certainly, it is a disease that has a very negative economic impact on the swine production, and its wide prevention, desired by the sector, has been promoting much academic and industrial research.

The great difficulty in achieving wide protection against *G. parasuis* lies in the intrinsic heterogeneity of microorganism different serovars, which makes it hard to develop an effective immunity and capable of preventing an infection process caused by serovars different from those contained in the vaccine formulation.

In Brazil, two commercial vaccines are available for the prevention of Glässer disease and, antigenically, they are composed of SV5 (Porcilis Glässer, MSD) (Segers et al. 2009) and a mixture of SVs 1 and 6 (Hiprasuis Glässer, HIPRA). The selection of these serovars to formulate these vaccines was based on results of epidemiological studies conducted in different countries, the capacity for homologous protection (animals vaccinated and challenged with a virulent strain and with the same capsular type as the vaccine strain) of these vaccines have already been demonstrated by different research groups, including ours (Frandoloso et al. 2011).

As already mentioned, in Brazil, cases of Glässer disease have already been associated with 12 different *G. parasuis* serovars and at least nine new capsular types not yet characterized. Considering that the protective response induced by usual vaccines is predominantly serovar specific, and on the other hand, assuming that there is cross-reactivity among certain serovars as described

by Bak & Riising (2002), a vaccine based on SV5 could potentially protect approximately 42.9 % and 44.8% of the clinical cases represented in figure 1 in the years 2019 and 2020, respectively. The heterologous protection coverage of a vaccine based on SV1 has not yet been experimentally demonstrated, so we can only assume its homologous protection potential, which could represent a protection coverage of approximately 13% of the clinical cases observed in Brazil.

When a commercial vaccine fails to protect vaccinated herds, the most appropriate solution, in a short term, is to develop autogenous vaccines, which need to be developed rationally. It is recommended to include in the formulation, strains isolated from systemic sites such as meninges, pericardium, and joints (Oliveira & Pijoan 2004; Smart et al. 1988). Primarily, it is also necessary to characterize their virulence profile. Thus, it is essential that laboratories producing autogenous vaccines carefully follow this premise and avoid as much as possible the inclusion of non-representative strains isolated strains of the trachea and lungs in their formulations. Another important aspect, widely observed in Brazil, is the formulation of vaccines combining pathogens that have low antigenic compatibility with each other. Often the pathogens used in the vaccine formulation cause the disease at totally different stages of production.

Regarding autogenous vaccines, it is crucial to typify and characterize the virulence of *G. parasuis* that will be included in the vaccine. Also, it is fundamental to define the genetic profile of strains belonging to the same serovar. Based on our experience, it is possible to find on the same farm and, even in the same pig, two genetically different strains belonging to the same serovar. In this case, it is necessary to formulate the autogenous vaccine with the two different strains, and failure to comply with this recommendation may compromise the desired protective effect of the vaccine.

Autogenous vaccines have gained prominence in the prevention of GD in Brazil. Although their long-term use tends to decrease as commercial vaccines with a broad spectrum of protection are launched on the market. In this sense, our group internationally has led the research for a vaccine composition that can promote protective immunity against all *G. parasuis* serovars. This goal is only achievable through the use of a structural, immunogenic, and conserved antigen within the "parasuis" species. Also, in this line, the TbpB protein presents itself as the most promising vaccine antigen for this agent.

Our studies have consistently demonstrated the homologous and heterologous protection capacity of the *G. parasuis* mutant TbpB protein (Barasuol et al. 2017; Frandoloso et al. 2011; Frandoloso et al. 2015; Guizzo et al. 2018; Prigol 2019). Furthermore, through an *in-silico* analysis, we demonstrated that a vaccine composed of three variants of the TbpB protein could prevent not only infections produced by *G. parasuis* but also infections caused by *Actinobacillus pleuropneumoniae* and *A. suis* (Curran et al. 2015; Guizzo et al. 2018).

8. Treatment of Glässer's disease

The choice and use of antibiotics during the treatment of clinical cases of Glässer Disease needs to be guided by the results of antimicrobial susceptibility tests. In Brazil, we demonstrate the circulation of clinical strains of *G. parasuis* resistant to several antimicrobial molecules routinely used in the swine clinic (Miani et al. 2017).

Recently, we evaluated the *in vitro* effect of Tildipirosin on 100 virulent clinical strains of *G. parasuis* isolated from swine from 6 Brazilian states (RS, SC, PR, SP, MG, and MS). The results of this work demonstrated that the therapeutic concentration of the product was effective in killing 90% of clinical strains (Peres et al. 2020). Furthermore, we demonstrated that the same efficacy was observed when treating animals experimentally infected with serovars 4 and 5 of *G. parasuis* (unpublished data). Thus, the use of Tildipirosin is recommended in the treatment of clinical cases of Glässer Disease.

Finally, we illustrate in **Figure 3**, the susceptibility profile of 394 clinical strains of *G. parasuis* isolated in 2020 in our laboratory. Of the antimicrobials evaluated, we warn that the strains were not very susceptible to Lincomycin, Sulfamethoxazole + trimethoprim, Tetracycline, Norfloxacin, Doxycycline, Marbofloxacin, and Tilmicosin. Conversely, Fosfomycin, Amoxycycline, Ceftiofur, and Florfenicol were the most effective antimicrobials in this assessment (AFK Imunotech, unpublished data).

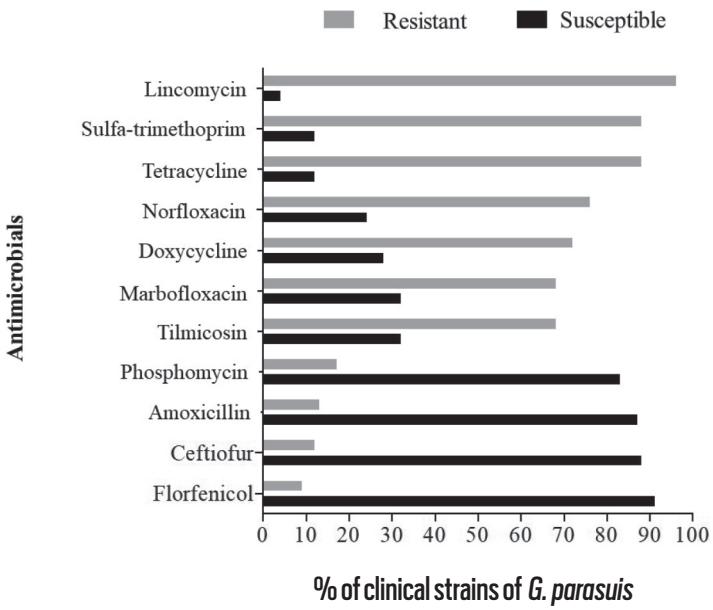

Figure 3. Profile of susceptibility and resistance of clinical strains of *G. parasuis*. Overall, 394 clinical strains were evaluated by the antibiogram test.

In general, these results highlight the importance of constant monitoring of the susceptibility profile of strains of *G. parasuis* circulating on the farm; to avoid the misuse of drugs and reduce the chances of the emergence of new resistant strains.

9. Final considerations

G. parasuis is a complex microorganism and capable of causing a systemic inflammatory disease known as GD that affects young piglets. Although there is limited information about the pathogenesis of the infection, today, we know that the agent can evade different immune responses to reach the host serosa. During episodes of GD, the isolation and definition of the *G. parasuis* serovar (molecular typing) are essential to establish a correct prevention program. Thus, the program can be based on commercial vaccines (when the serovar present on the farm is present in the vaccine formulation) or autogenous (only when the serovar present on the farm is not present in any commercial vaccine). The serological diagnosis can be used strategically to correctly implement the immunization protocol to reduce the piglet's susceptibility window to GD during the nursery phase. The future of preventing infections produced by *G. parasuis* is conditioned to the development of modern vaccines and with broad heterologous protection.

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Strategies for the prevention, detection and management of infection by the PRRS virus (and other pathogens)

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Highlights:

1. A powerful toolbox that includes efficacious strategies, materials, and methods has been developed for the prevention, detection, and management of PRRSV infections.
2. Most of these tools can be used to control other endemic pathogens currently in circulation in Brazil.
3. As far as we know, several of these tools are not currently being implemented in many of the Brazilian swine operations. Thus, the authors strongly encourage managers and veterinarians to consider implementing these tools. This would allow the preparation for infection by the PRRSV and other emerging agents in global pig farming, while at the same time bringing benefits in the efficiency of pig production in the short term.

Introduction

This manuscript is a summary of the material presented by our group at the FarmTalks online conference, presented on August 12, 2020, from Farmabase studios in Brazil. The main objective was to summarize and highlight the central strategies and tools that were developed to manage porcine reproductive and respiratory syndrome virus (PRRSV) in swine populations. The secondary objective was to illustrate that several of these strategies, although developed in response to PRRSV, have been adapted and implemented for other pathogens, including *Mycoplasma hyopneumoniae*, Senecavirus A (SVA), and porcine epidemic diarrhea vírus (PEDV).

As well as the presentation, this article is organized into the following sections: considerations regarding PRRSV, prevention, diagnosis, epidemiology and control, and the situation in Brazil.

Considerations about PRRSV

PRRSV is a virus of the Arterividae family that affects only pigs. It is present in all countries with globally relevant pig production, with a few exceptions such as Brazil.

PRRSV is sub-divided into two species, PRRSV-1 also known as a species of European origin, and PRRSV-2, also known as having a North American origin. Both species have global circulation, although they have some exceptions, as in Spain, for example, a recent article conducted in one of the largest pig production systems reported the detection of PRRSV-2 only (Torrents et al., 2019). Vaccination studies using live attenuated virus report some cross-protection of PRRSV-2 against PRRSV-1 but not vice versa.

The economic impact of PRRSV in the USA has been estimated at $ 250 *per* sow, $ 2-20 *per* feeder pig, or approximately one billion dollars a year in that country (Holtkamp et al., 2013).

PRRSV is one of the most mutagenic viruses known, evolving at a faster rate than Influenza or acquired immunodeficiency syndrome (AIDS) viruses (Jenkins et al., 2002; Hanada et al., 2005).

The viral shedding can be detected from oral fluids, semen, secretions, and respiratory aerosols. Transmission can occur by direct and indirect routes, the first being horizontal and vertical. Both pathogenic and attenuated strains have a high potential for transmission in susceptible populations. Similarly, the clinical manifestation of PRRSV infection depends on several factors that include the herd's immunity level, the virulence of the viral strain in question, and other stressors. In other words, PRRSV infection is possible (and frequent in endemic populations) without evident clinical manifestation. On the other hand, the introduction of highly pathogenic strains in naïve herds can generate severe clinical impact like significant reproductive losses, as well as delayed growth performance and increased mortality rates for all ages.

One of the main characteristics of PRRSV replication is that this process occurs mainly in macrophages in the lungs, which has a significant impact on the ability of the immune system to respond effectively to other health challenges. Therefore, there is a considerable synergy in the infection by PRRSV and secondary agents, including the Influenza A virus, *Mycoplasma hyopneumoniae*, or *Streptococcus suis*.

Prevention

The purpose of this section is not to present a complete review of biosafety aspects and practices, but to highlight the biosafety aspects that have been developed and/or improved in response to PRRSV. It is important to note that most of these strategies have been shown to be effective in preventing various

other infectious agents.

Considering that PRRSV, like other viruses, has a relatively high infectivity half-life (that is, it remains infectious for a long time in the environment) at low temperatures, it is crucial to ensure that trucks used for transportation are properly decontaminated between uploads. It is a consensus among experts that transport biosafety is a pivotal factor in preventing PRRSV. Factors related to transport biosafety include procedures for washing and decontaminating the trailer and cabin, procedures to ensure complete post-wash drying, either by natural means or by using fans. For vehicles transporting high-value pigs, such as breeding pigs, the use of forced hot air (thermo-assisted drying and decontamination - TADD) is usual to reach high temperatures (70-72ºC) for 20 to 30 minutes, ensuring virus inactivation. According to Dr. Gustavo Simão, TADD units have been successfully implemented in Brazil. Other points on transportation biosafety are: ensuring that the driver remains in the cabin (that is, without contact with the charterer or any access to the farm); the use of pig transport routes based on health pyramids; and having a fleet of carts dedicated to 'clean' pyramids - that is, carts that have access to PRRSV positive farms (that is, shedding viruses) should not have access to negative farms.

Another important point on biosafety against PRRSV is the concept of layered biosafety where multiple 'zones' are implemented to minimize the risk of virus transport between dirty and clean areas. A simple example is the use of zoning during the procedure for loading weaning piglets, where instead of a simple division between dirty area/clean area, one (or multiple) transition area (s) with employees and utensils dedicated to this (those) zone(s) is(are) implemented. At the end of the process, the transition zones can be decontaminated starting from the closest to the animals to the most distant. Other important concepts include efforts to implement unidirectional flows (people and animals), a minimum number of origins, use of internal replacement ensuring self-sufficiency of genetic replacement), sanitary acclimation of gilts as soon as possible before the introduction into the reproductive herd (ideally at least 90 days before); the use of quarantine, introducing animals into the herd after confirmation of a negative status by diagnostic tests; and use of biosafety scores to help understand the degree of vulnerability of introducing pathogens into the herd (Silva et al., 2019).

Diagnosis

Serology is a method widely used mainly to screen presumably negative populations (to confirm such status) for PRRSV. Seroconversion occurs between 7 to 14 days after infection and detected by using commercial kits such as IDEXX kits. In cases of unexpected positive results, confirmation is achievable by IFA or IPMA. Sampling for serology can be done on animals of all ages, using processing fluids, oral fluids, or blood serum.

The detection of viral RNA is performed by the RT-qPCR test, detecting viremia in blood or processing fluids, or shedding in family oral fluids, oral fluids from growing pigs, slaughter pigs, or breeding pigs, or in the air (Prickett et al ., 2008; Almeida et al., 2018; Lopez et al., 2018; Almeida et al., 2019; Trevisan et al., 2019a; Trevisan et al., 2019b; Trevisan et al., 2020). The RT-qPCR technique can also be used to detect virus-carrying status (that is, an infected but non-viremic, non-shedding animal) in tonsils, lungs, or lymph nodes. The detection of viral circulation in farrowing units is commonly done by using processing fluids, family oral fluids, or serum from suckling piglets.

Production data aggregated daily or weekly can also be incorporated into the PRRSV monitoring plan, complementing the diagnostic results (Silva et al., 2017), which can be done manually or automated. Aiming to detect significant changes in productivity indicators, which are typically altered due to PRRSV, such as the number of abortions, prenatal mortality (mummified and stillborn), the mortality of suckling piglets, or growth performance indicators such as daily weight and mortality rate from weaning until slaughter.

Field epidemiology tools for the control or elimination of PRRSV

For both the control and elimination of PRRSV from swine heard, it is essential to adopt strategies to minimize the circulation and spread of the virus within the herd, such as the use of unidirectional pig flow and the use of herd immunization strategies. Among the most used tools, we highlight the use of internal biosafety measures (that is, biocontainment), preventing the spread of the virus between cages, rooms, and sheds; the use of mainly attenuated vaccines to generate homologous immune protection (ideally) in the herd; the temporary closure of the farm, that is, temporary interruption of entry of replacement animals; and the flow of gilts ensuring the entry of immune animals and without viral shedding in herds that are in the process of virus control/elimination.

As with any project, PRRSV control or elimination programs must be closely monitored using appropriate metrics. Some metrics we use, ensuring process management and comparison of different tools in field studies are: the proportion of success in eliminating the virus within a pre-agreed period (e.g., one year); time to consistently produce PRRSV negative piglets; time to recover the level of productivity that the farm had before the viral infection; total losses calculated by piglets weaned below the expected, from the infection until recovery of normality; and cost analysis: benefit of the control/elimination program. In general, attenuated vaccines, especially in combination with temporary farm closure are appropriate tools for eliminating the virus from infected farms. Attenuated vaccines are also efficacious in reducing pneumonia and reducing the impact on pig growth. Many veterinarians choose to have preventive vaccinations on farms with

frequent outbreaks (every 2-3 years or more frequently). Many veterinarians also use the vaccine in response (i.e. "therapeutic" use) to outbreaks.

The table below summarizes our general recommendations for the control and elimination of PRRSV from infected populations.

Reality Brazil

In Brazil, considering the high frequency of continuous pig-flows, natural ventilation, mixing animals from different origins, the presence of growing pigs on farms where there are also breeding stock, and low adoption of early gilt acclimation programs and quarantines, we believe that an eventual introduction of PRRSV in the country would result in a significantly more prominent economic impact than that in the USA. As a result, it is vital that Brazil continues to join efforts to avoid the introduction of PRRSV, although several neighboring countries are positive.

There are many tools and concepts developed for PRRSV that serve, in most cases and with minor changes, for PEDV, *Mycoplasma*, Seneca, Rotavirus, *Salmonella, Brachyspira*, *Lawsonia,* and other agents. Therefore, we raise the question for leaders and pig farming managers in Brazil: why not implement these concepts and strategies today, preparing for the major challenges of the future, while improving the efficiency of production and culture of health/biosafety in the short term?

We believe that with the increase in the size of the pig populations (farms, production systems), increased global competitiveness in pig production, and increased diversity of pathogens, the value of health only tends to increase over time differentiating those who make a profit from those who have losses. As a result, investment in sanitation pays for itself in the short and long term through better production efficiency.

Factor	Target, route to elimination	Target, route to control
Viral circulation (prevalence)	Zero	Low
Type of PRRS virus	From field to none	From field to MLV
Replacement gilts	Naïve when prevalence reaches zero	Previously immunized (2-3 months), without shedding
Semen	Naïve	Naïve
Piglet vaccination strategy	Depends on the probability of infection and severity of PRRSV in the region *	Depends on the probability of infection and severity of PRRSV in the region *

* Ranging from multiple doses from the first week of life (situations of a high challenge) to no vaccination (negative piglets in free regions).

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02

Enteric Health

Rotavirus: How to control the different serotypes - North American experience

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Introduction

Rotaviruses are a common cause of neonatal and post-weaning diarrhea in pigs worldwide.[1] Diarrhea is primarily a malabsorptive mechanism via viral destruction of the apical enterocytes and resultant atrophic enteritis, but there is also a secretory mechanism through the release of a viral enterotoxin.[1,2] Clinical diarrhea with rotavirus results in reduced weight gain (225-635 grams at weaning), increased treatment costs, morbidity and mortality (3-20%).[3-5] The majority of losses occur in young suckling piglets less than 1-2 weeks of age, however post-weaning diarrhea can be significant, especially if co-infections or other stressors are present. While rotaviruses in swine have been known about for some time[6], recent diagnostic developments (multiplex PCR, sequence analysis) have highlighted the prevalence and diversity in modern swine production.[2,7] Rotaviruses commonly seen in swine are types A (RVA), B (RVB) and C (RVC), and while E and H have been documented, these types are less prevalent.[1] Anecdotally, RVC appears to be the most frustrating to veterinarians with variable success with control measures.[3,8,9] RVB appears to be less prevalent and not normally the focus of presentations or discussions on rotavirus control. There is a general assumption that RVC is more prevalent pre-weaning and RVA more prevalent post-weaning, however RVA, RVB and RVC can be seen at any age in pigs[2] and will depend on herd immunity, population dynamics and environmental exposure. It is important to note that individual pigs can be co-infected with multiple types (RVA, RVB and RVC) of rotaviruses[10], and likely within type co-infections (i.e., multiple G types) also occur.

Challenges

Rotaviruses are non-enveloped double stranded RNA viruses, with a genome of 11 segments, which allows for recombination and increased

mutation rates.[7,11] There is no immunological cross-protection between serotypes RVA, RVB and RVC, meaning control must be independently considered and addressed for each. Within types, there is further genetic diversity of the immunological epitopes; the outer capsid proteins VP7 (G group) and VP4 (P group), which induce neutralizing immunity.[7] Variation within rotavirus groups (A, B, C) at the G and P types, generate significant diversity and limit cross protection between isolates. Rotavirus has been referred to as the "influenza of the gut", due to the above characteristics, and control relative to vaccination likely has the similar challenges to controlling influenza in swine.[11] Two other epitopes of interest are the more conserved inner capsid VP6 (defines the A/B/C type), which produces cross-reactive antibodies within group only[11], and the nonstructural glycoprotein NSP4, which acts as a viral enterotoxin resulting in secretory diarrhea.[12] More work is needed on the clinical effectiveness of immunity to these later epitopes, but may allow for more broad protection against rotaviruses if used in combination with VP7 and VP4 epitopes.

One of the biggest limitations with advancement of rotavirus knowledge and control is that only RVA grows readily on cell culture, while RVB and RVC are much more difficult to grow. This limits the development of research, diagnostic tests and commercial vaccines. The only commercial rotavirus vaccine available for swine in North America is a RVA-based modified live virus (ProSystem Rota line, Merck Animal Health, Madison, NJ). It has been possible, although difficult, to develop limited autogenous RVC vaccines using conventional methods or the RNA particle (RP) technology platform (SEQUIVITY, Merck Animal Health, Madison, NJ). However, the effectiveness of these vaccines has been only recently evaluated and data is limited.[4,5]

The development of diagnostic tests has been difficult due to the fastidious nature of RVB and RVC. While we only recently have tests such as multiplex PCR [7] and *in situ* hybridization[13], there is still a lack of commercial tests for other useful tools such as, type-specific serology and immunohistochemistry (only RVA IHC is available). Serological tests for RVA and RVC are currently limited to research in small volumes. Recent work by Chepngeno *et al.* utilized an ELISA made from RVC virus-like particles, that could be beneficial if commercialized.[14] While these tools may not add significantly to the diagnosis of rotavirus in the field, they would serve to help us better understand the epidemiology and effectiveness of control measures.

Control

Control of rotavirus is aimed at environmental sanitation, providing adequate levels of maternally derived (passive) immunity through colostrum and milk (lactogenic immunity) and management strategies that maximize and maintain adequate ingestion of the immune constituents.[6,14] Methods to

improve lactogenic immunity include exposure of the dam to the pathogens in some form ("feedback", vaccination, etc) at some time prior to farrowing, with enough time to develop antibodies (particularly IgA and IgG; namely targeting VP7 and VP4) in the milk that neutralize the rotaviruses and prevent viral entrance and damage to the enterocytes.[15,16] Exposure late gestation, commonly in the 6 to 3 weeks prior to farrowing, is assumed to be a booster of previous exposure although that is often not definitively known. As with many swine diseases, rotavirus appears to be more of a challenge in piglets/litters from gilts, which is very likely due to the gilt's immunological immaturity and lower quality of lactogenic immunity, as compared to older parity females.[14]

Sanitation

As with most diseases, and especially enteric pathogens with fecal-oral transmission, sanitation becomes important in controlling disease through reducing environmental load and challenge dose. Any immunity transferred to piglets in milk, can be overcome by extremely large doses of pathogen. In farrowing barns, crates should be thoroughly cleaned between use, with all-in/all-out farrowing by rooms as the standard, if possible. Sanitation should include use of a detergent/descaler, hot pressurized water, disinfectant and surfaces allowed to completely dry. Disinfectant selection should be considered to address the pathogens types of most concern for the farm. Rotaviruses, being non-enveloped, would be most sensitive to aldehydes and sodium hypochlorite (bleach) followed by alkalis and peroxygens. Antimicrobial spectrum of common disinfectants can be found at: [http://www.cfsph.iastate.edu/Disinfection/Assets/AntimicrobialSpectrumDisinfectants.pdf] It is important to note that many disinfectants have reduced activity in the presence of organic material, therefore thorough cleaning of surfaces prior to disinfection is paramount. Allowing surfaces to completely dry allows the pathogens to be killed by desiccation, however in many modern production systems, time is a commodity and pig flow may not allow adequate dry time. In this case, adding heat or desiccants to the surfaces may be beneficial, but need to be evaluated for effectiveness.

Vaccines

Rotavirus type A commercial modified live vaccines (ProSystem Rota/RCE, Merck Animal Health, Madison, NJ) exist for swine and are generally reported to be effective when implemented. The general efficacy of the vaccine is likely related to the modified live nature of the vaccine, which would actively replicate and stimulate enteric immunity in the animal. There are two G types

(G9 and G5 based on sequence analysis) included in the vaccine, so vaccine failure may be due to a G-type mismatch in a particular farm or system.

Part of the challenge and frustration with RVB and RVC is the difficulty to isolate and adapt to cell culture, thereby precluding the development of conventional vaccines. Therefore, non-traditional methods of vaccine development, such as RNA particle, bacculovirus expression or virus-like particles is necessary, but these methods can be expensive and limit the immunogenicity of the vaccine. To date there have been limited reports of the effectiveness of vaccines targeted against RVC,[4,5,17] however development of vaccines effective for multiple rotavirus types would be beneficial in removing the negative aspects and risks of natural planned exposure ("feedback") discussed below. More work needs to be done to demonstrate the effectiveness of the different vaccine methodologies.

It should be noted that use of a killed vaccine or technology that does not result in similar immunity to live infection, very likely does not product good immunity as a stand alone protocol. May of these vaccine strategies will require animals to be exposed to live virus, through natural or planned exposure, and then boostered with the vaccine at a desired time prior to farrowing to maximize immunity.

As explained above, rotavirus are genetically diverse and vaccine isolate selection is important. Isolate selection for a vaccine must take into account both the serotype (A, B, C) as well as the G (VP7) and/or P (VP4) types. This becomes more important and challenging when developing a vaccine for multiple farms or system. Research would suggest that both G and P are important epitopes for neutralizing immunity, however vaccines may only contain G type epitopes. With sequence analysis we can determine overall percent nucleic acid and amino acid (AA) similarities between isolate epitopes but there is very little information on what specific AA changes influence clinical immunology. As a loose guideline, 95% AA similarity or above is the currently utilized level to predict clinical cross-protection. Due to these issues, it is the author's belief that successful use of a rotavirus vaccine incorporates some level of isolate surveillance and sequence analysis comparisons.

Natural Planned Exposure (*Feedback*)

Due to the frustration with RVB and RVC control, and lack of conventional vaccines, several creative methods have been developed to control rotavirus.[3,8,9,18-20] The purposeful exposure to any combination of diarrhea, manure, tissues to breeding stock, commonly known as "feedback" or Natural Planned Exposure (NPE), has been long used as a control measure for enteric and systemic pathogens on swine farms.[21] The main concerns with this method are: 1) the temporary control and "roller-coaster"[22] effect often seen

when infectious material is no longer available when the disease is controlled and 2) the inconsistent quality and unknowns of what pathogen are or are not in the "feedback" material.[23]

In an attempt to better control the "roller-coaster" effect, some practitioners have improved upon the above process by freezing larger amounts of the "feedback" material on farm. While this helps to extend the life of the "infectious" material across more breeding groups, it still does not address the issues of unknown pathogens nor consistency between batches.

The next step in the evolution of the "feedback" process was the commonly referred to "ice cube" method, whereby colostrum deprived (CD) pigs are given material known to be positive for the desired rotavirus (es), allowed to replicate virus naturally over a 24 hour period, then euthanized and intestinal contents and/or intestinal tissue harvested, diluted and frozen for future use in a "feedback" exposure. The intestinal homogenate is commonly aliquoted into ice cube trays as a convenient method of freezing equivalent volumes, hence the *nome de plume*.

In an attempt to improve upon the above "ice cube" method, the author has previously reported on a modified protocol, whereby the process was done at a system level, in tiered fashion across multiple farms with common RVA and RVC, termed the "Master Seed" method.[20] The NPE material is methodically tested for key pathogens (Rotaviruses, PRRS, PCV2, PCV3, coronaviruses, bacteria, etc) and either Passed or Failed prior to use of the frozen material on each farm. While an assumed improvement over the above methods, the "Master Seed" method still has similar drawbacks in that 1) rotaviruses continue to mutate and updates to isolates may be needed at the system or farm level, 2) one can never screen for every possible pathogen, 3) the process is labor intensive and sacrifices piglets, 4) the process of NPE can still be variable between farms and 5) NPE batches are still inherently highly variable within and across farms. Advantage to this process is the ability to standardize the process across multiple sites and better control the exposure dose and volume given to animals.

Recent work suggests that number of exposures and timing of NPE may be important aspects of optimizing control of rotavirus.[4,24] This is supported by other work done on porcine epidemic diarrhea virus (PED)[25] which likely relate to the mechanisms of swine enteric viral maternal-piglet immune axis in swine. Work with PED demonstrated that a higher level of immunity was transferred from dam to piglet when an initial exposure was given to naïve gilts mid-gestation as compared to late gestation, however it is not clear if boostering previous immunity would have the same gestational-stage related impacts. Some unpublished work with killed PED and rotavirus vaccines in previously exposed animals would suggest that boostering immunity as late as 1 week prior to farrowing provides significantly improved clinical protection.

Caution should be taken as exposure late gestation may increase shedding in gilts entering farrowing and increase environmental load.

One limitation of NPE is that an effective challenge dose for pregnant sows is not known, and it is likely that the amount of live virus required to stimulate or booster immunity is quite high, especially in previously exposed and immune animals.[21] In addition, targeted dosing is limited to PCR cycle time (ct) values, which does not equate to live infectious virus, but is the only current measure of viral load for rotaviruses. Current volumes and doses of NPE material to use in sows appears to be based solely on individual veterinary experience.

Combination measures

It is likely with the currently available tools and challenges that a combination of the above strategies will be required for successful control of rotaviruses on farms. It is the opinion of the author that replacement breeding stock need to be purposefully exposed to endemic live rotaviruses at entry into the farm, prior to breeding. Followed at some time in gestation with a killed vaccine containing similar epitopes to booster the immunity that will be transferred to piglets thorough their milk. There are still a lot of questions as to the methodology to optimize this protocol, but adjustments can be made as that data is available. In the meantime, control what you can control.

Post-weaning Rotavirus

To date, focus in North America appears to be solely on the control of rotavirus in the suckling period. Control of rotavirus post-weaning has limited reports or research published. It is the author's experience that a very large proportion (close to 100%) of pigs are infected with multiple rotaviruses immediately post-weaning, consistent with the loss of lactogenic immunity. In a study that followed a subset of pigs from birth to 3 weeks post-weaning, 100% percent of pigs tested positive by fecal PCR for RVA, RVB and RVC 1, 2 and 3 weeks post-weaning. It would be valuable to understand if early exposure to RV (<14 days of age) produces immunity that would provide protection post-weaning (>3 wks of age) and reduce the production losses (gain, morbidity, mortality) associated with rotavirus. It would make sense that better control of rotavirus during the lactation period, might increase challenges with rotavirus post-weaning, due to the limited exposure and active immune development of the piglets. However, pigs less affected early in life tend to be more robust post-weaning and therefore relative impact from rotavirus would be less as the pig ages.

Future Work Needed

There is still a lot to learn about rotavirus, especially RVC, in swine. It is the opinion of the author that the below areas should be explored to provide valuable information to veterinarians and farmers to better control rotavirus.

Veterinarian, farmers and animal scientists need to continue to evaluate and communicate the impact of rotaviruses in their farms, herds and systems. Studies that evaluate the production loses and economic impact will help the industry characterize the opportunity and assist researchers secure funding for the more essential research discussed below.

We need to continue to develop and provide commercial serology and milk-based antibody tests. This will allow researches and field veterinarians to evaluate herd immunity and determine if control measures are actually stimulating effective immunity. These tests would also benefit the below studies.

We need to determine the correct or effective challenge dose of rotaviruses are to adult, previously exposed females. A known effective dose would help veterinarians establish the level of material needed for exposure protocols, without wasting excessive product and resources. The effective challenge dose may be different for RVA, RVB and RVCs, and may be influenced by level of immunity already in the animals. Based on experience and limited data[5,26], it is the author's opinion that effective dose of a NPE material must have a rotavirus PCR ct values of 20 or less for RVA, RVB and/or RVC. For example, the author's current NPE mixing rate is an initial 0.4 mL of NPE is used per animal to be exposed, but diluted first into a volume of water (~1%: 0.4 mL NPE + 39.6 mL water) to be sprayed or feed/water slurry (1/2 cup) to be fed in a way convenient for farm staff to deliver effectively.

More work needs to be done on the optimum timing of exposure and/or vaccination for maximizing maternal immunity in previously exposed animals and/or naïve animals.

As with influenza, understanding the specific amnio acid changes within an epitope for rotavirus, and how those relate to immunological cross-protection within G, P and serotypes would be beneficial in predicting when vaccines would need to be updated, or which isolates would provide the broadest protection to circulating isolates in a farm, system, region or county. It is very early in the clinical analysis of sequence data for rotavirus.

With the relative efficacy of the modified live RVA vaccine available, continued work on adapting RVB and RVC to cell cultures that would allow for manufacturing into a multi-valent modified live vaccines would be beneficial. Multivalent modified live vaccines could be used to evaluate efficacy in preventing or mitigating the impacts of rotavirus in pigs post-weaning.

One area that is not well understood is the epidemiology of rotaviruses in breeding herds. Subpopulations may exist in herds and result in sustained

endemic disease. It is highly likely that incoming breeding stock are infected sub clinically and bring in rotaviruses to sow farms. If incoming strains are similar to resident strains then endemic disease may persist, but changing gilt sources may result in new dissimilar rotavirus strain(s) entering a herd, and thus result in epidemics of disease are likely to occur.[3,17]

Summary

Rotavirus continues to be a predominant pathogen identified in suckling piglet diarrhea worldwide. Complication in controlling the disease is mainly due to the antigenic diversity and fastidious nature of the rotaviruses, limiting the diagnostic and immunological techniques available to researchers and veterinarians. In addition, compared to other swine pathogens, there is limited work being conducted on rotaviruses. As a result, veterinarians and farmers have become frustrated with rotavirus control and have developed unique techniques to attempt to control the disease with varied results. Newer technologies are now becoming available that will allow us to better understand the diversity of rotaviruses, evaluate the epidemiology and manipulate immunity to control rotavirus and subsequent clinical disease.

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Strategies to Prevent and Control Proliferative Enteropathy (Ileitis)

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The swine industry has the information and tools today to try to either prevent ileitis in sow herds or pig-flows and to also eliminate it from currently positive systems. As swine herds eliminate economically important disease pathogens such as PRRS, PEDV, and M. hyo, ileitis should be the next economically important endemic bacteria to tackle. Veterinarians must first understand and be convinced of the significant cost of both clinical and subclinical ileitis and be willing to monitor pig-flows to be sure neither form of the disease exists.

Our current control programs with vaccine or antibiotic pulses, although very cost effective, do not prevent subclinical infection and are never as good as no infection at all. In randomized controlled *Lawsonia intracellularis* challenge trials with vaccines and/or antibiotics, this is proven time and again. The strict negative control groups (non-challenged, non-treated) almost always have significantly better performance than the vaccinated or antibiotic groups (treated, challenged).

Four Key Elements. There are four key elements ("tools") swine vets must understand and apply to successfully prevent and/or eliminate ileitis from a production site or system:

1. Antibiotics – Carbadox is a unique antimicrobial compound that can be used to both prevent *Lawsonia intracellularis* colonization and also eliminate *Lawsonia* carriers when fed for at least 14 days at 50 g/ton.[1,2]
2. *Lawsonia* Immunity - *Lawsonia intracellularis* is a unique intracellular bacterium that lends itself to elimination because once exposed, a pig obtains potential life-long immunity. Pigs are resistant to re-infection after initial exposure to low or high doses of the bacteria.[3] This knowledge can be useful to eliminate both subclinical and clinical infection in sow herds, replacement gilts, and grow-finish pig-flows with controlled live exposure followed by an antibiotic pulse.[4]
3. Diagnostic Tools for Analysis – Ante-mortem fecal PCR's and IPMA serology both are useful to determine the ileitis status of a herd and differentiate ileitis vaccination vs infection titers.

4. Biosecurity – Needless to say, only herds with very good biosecurity will remain free of ileitis. Ileitis is not known to be spread through the air or the feed, so it should actually be easier to prevent than some other diseases.

Preventing Ileitis with Carbadox. "Prevention" here means not having subclinical or clinical *L. intracellularis* exposure at all, i.e. negative fecal or oral fluid PCR's.

Although it does not have FDA approval for ileitis treatment or control, it has been thoroughly studied in randomized controlled ileitis challenge studies. It is the only known antimicrobial compound that when fed at 50 g/ton for 14 days eliminates *Lawsonia* from clinical or subclinical carriers.[1] It is also the only antimicrobial that prevents colonization in pigs challenged with low or high doses of *Lawsonia intracellularis*.[2] There are five FDA approved antibiotics in the feed that effectively control and treat clinical ileitis. These antibiotics typically will not prevent *Lawsonia* colonization or eliminate the carrier pig. However, at higher antibiotic levels and/or longer treatment times, these antibiotics may also prevent colonization or eliminate the Lawsonia subclinical carrier pigs. Further research in randomized controlled trials will need to be done to determine this.

Wean-to-Finish Example – In a large pig-flow with 8 separate sites of 4,000 to 10,000 pigs, potential subclinical weaned carriers are treated with Carbadox 50 (and OTC 400) in the Nursery 1 and Nursery 2 diets for about 17 days post-arrival. This gives us confidence *L. intracellularis* is not "leaking" from the sow farm in subclinical carriers. In three of those sites, we do not vaccinate for ileitis because they have excellent biosecurity and historically have not had any bacteria present. In the other sites we do vaccinate for ileitis to get enhanced immunity in case of exposure to *Lawsonia*. All sites are monitored via fecals and/or oral fluid PCR's at 120-280 lbs B.W. for the presence of *Lawsonia* to be sure the programs are working.[5]

Sow Herd Prevention – Similarly, preventing ileitis from establishing itself in a new or repopulated sow site on subclinical carrier gilts could be achieved by having Carbadox at 50 g/ton for at least 14 days in the gilt diets prior to moving them into the new or cleaned facilities. Once a negative sow herd is established, replacement gilts will need to be monitored via fecal PCR's to be sure subclinical carrier gilts are not present.

Establishing ileitis free sow herds and/or monitoring replacement gilts to ensure a negative *Lawsonia* status could and should be done in breeding stock multipliers. Once a sow herd is established negative it will be providing a weaned pig free of *Lawsonia*. Subsequent downstream pig flows can also be established free of ileitis. This could be very beneficial in antibiotic-free or NAE niche markets.

Ileitis elimination in sow herds can likely be accomplished with the same antibiotic regimes used to eliminate Swine Dysentery (*Brachyspira hyodysenteria*) from sows herds. For example, a 6 week treatment of 200 g/

ton tiamulin with proper sanitation, rodent control, and a closed herd that has been proven to eliminate SD should also be effective for *Lawsonia* elimination. Swine veterinarians need to apply and test this hypothesis.

Elimination in grow-finish pig-flows with controlled *Lawsonia* exposure and antibiotics

In all finish pig-flows, the first goal for ileitis control programs is to not ever have any clinical signs of ileitis. Clinical ileitis will cost $10-$22 per pig in lost performance, mortalities, and treatment costs depending on the clinical severity.[5] The second important goal is to not have any subclinical ileitis – i.e. no positive *Lawsonia* fecals in the grow-finish stage. This can be accomplished with the DLI^R Immunity Program.

DLI^R (Diluted *Lawsonia intracellularis*) Immunity is a patented method of administering a specific controlled dose of oral live *Lawsonia* for enhanced immune stimulation compared to commercial vaccine. The program requires an autogenous source, VCPR oversight, and is followed up with an antibiotic pulse two weeks post-DLI^R. When done properly, the pigs are completely resistance to colonization and subsequent bacteria fecal shedding found in both subclinical and clinical ileitis.[3,4]

DLI^R Immunity live exposure is used to eliminate both subclinical and clinical ileitis and not have any fecal shedding at all or any clinical signs of diarrhea, or positive fecal color scores in grow-finish pigs. It is a good tool to use in problematic ileitis barns or sites.

The Enterisol^R ileitis vaccine has been shown to reduce clinical disease and to increase weight gain. However, while the natural infection with *L. intracellularis* can provided complete protection against re-infection, this has not been achieved by this vaccine. Cell-mediated immune responses are likely mediators of protective immunity against *L. intracellularis*, with CD8+ effector cells, and CD4+, CD8+ positive memory T cells as main contributors to the antigen-specific IFN-y production.[6]

Elimination in Replacement Gilts with Natural Exposure

A likely source of *Lawsonia* exposure into sow herds is from asymptomatic subclinical replacement gilts. These gilts should be monitored via fecal PCR, oral fluids, and/or serology to be sure they are not shedding *Lawsonia* prior to entry into a sow unit. Occasional PHE (Porcine Hemorrhagic Enteropathy) outbreaks occur in ileitis vaccinated gilts and also rarely in multiple parity sows. This could be prevented with the DLI^R Immunity Program in replacement gilts followed by antibiotics 14 days later. This system works

well in gilt isolation barns with AI/AO pig-flow. Eventually the entire sow herd would have complete *Lawsonia* protection as normal sow cull rates are replaced with ileitis immuno-protected gilts. This example has been used in a "high-health" commercial client for about 8 years which supplies ileitis negative pigs for research trial work.[5]

Antemortem Diagnostic Monitoring

Lawsonia PCR's are useful in a number of applications:
• Ensure negative fecal shedding in weaned pigs from negative sow herds
• Monitor AIAO gilt pools to be sure they are no longer shedding after post-DLI^{R} immunity and an antibiotic pulse (e.g. Carbadox, tiamulin, etc.)
• Monitor finish pigs post-DLI^{R} to confirm no subclinical (or clinical ileitis) is present
• Determine the PCR presence and quantity of *Lawsonia* in fresh fecal samples or oral fluids

In general, fecal PCR's are a very useful diagnostic tool to be sure any mild diarrhea or fecal blood is *Lawsonia* positive or negative. If the ct value at the U of MN is < 31 ct 40, the sample is associated with PPE intestinal lesions and economic performance loss.[7]

Oral Fluid PCR's - Oral fluids are also useful to determine the presence and quantity of *Lawsonia* in the environment. The ct values are interpreted the same way as with fecal PCR's and have similar or slightly lower number values than with pooled fresh fecal PCR's. One advantage is that more pigs are usually chewing on the ropes so sensitivity may be increased.

Serology – IgG *Lawsonia* antibodies measure prior exposure to either field infection or *Lawsonia* vaccine. Know what to expect for *Lawsonia* titers relative to the antibody test. For example:
• Unvaccinated, negative herds will not have any antibody titers
• Porcilis^{R} and DLI Immunity^{R} will have < 480 titers on IPMA
• Enterisol^{R} oral vaccine does not illicit an ELISA titer response at all and an inconsistent IPMA titer response[8]
• Field exposure or ileitis outbreak titers are usually > 480 and much higher (up to 15,000) depending upon the disease insult.

More on Biosecurity. Knowledge regarding the epidemiology and transmission of *Lawsonia intracellularis* is imperative to prevent it from contaminating a clean site. Although there are still plenty of knowledge gaps, we know transmission is primarily through asymptomatic carrier pigs, and fomites (people, trailers, equipment, etc.) in contact with contaminated feces. *Lawsonia* can live in manure and pits for at least two weeks, probably longer. Cleaning and disinfection procedures should include a detergent step, hot water wash, and disinfection. It has been proven that insects (flies, cockroaches) and rodents are mechanical and biological vectors for *Lawsonia* transmission, respectively.[9]

Summary

It is always more economical to prevent and eliminate diseases than to treat and/or control them. The swine industry now has both the knowledge and tools available to prevent and/or eliminate *Lawsonia intracellularis* from pig-flows. Swine veterinarians need to begin to establish *Lawsonia* free pig-flows and systems and monitor their success to motivate others to do the same.

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Difference between Colostrum and Lactogenic Immunity: Practical Enteric Immunity

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Introduction

All sow farms have a common goal: to consistently produce a high-quality piglet in an efficient and profitable manner. Consistency is critical as it helps those who end up feeding these piglets out to market (slaughter or breeding stock sales). Efficiency is critical as farm labor availability is becoming more and more of an issue worldwide. Finally, profitability is of the upmost importance as ultimately it is the driving force for the existence of any industry.

Enteric diseases are some of the most significant contributors to baby pig morbidity and mortality in the farrowing house. The latest U.S. National Animal Health Monitoring System (NAHMS) Swine Report data from 2012 indicated that 60.6% of breeding herds had problems with navel infections and 47.8% had problems with colibacillosis. Post weaning, 65.2% of herds reported having issues with *Streptococcus suis*, 46.6% with Porcine Reproductive and Respiratory Syndrome, and 32.4% with colibacillosis. Especially immediately post weaning, enteric challenges are well recognized by the swine industry as pigs adapt to new environments and transition from a liquid diet (milk) to solids (ground feed). Once in the finishing phase, the primary respiratory (PRRS, Influenza and *Mycoplasma hyopneumoniae*).

With the introduction of porcine epidemic diarrhea into the United States, swine veterinarians were reminded of the difference between colostrum and lactogenic immunity. It is the goal of this paper to briefly review key concepts on maximizing piglet immunity through both colostrum and lactogenic immunity. The goal is to provide relevant and practical tips that will help sow farms achieve a consistent goal of producing high quality pigs efficiently and profitably.

Piglet Immunity

Weaning weight is considered one of the most important factors impacting post-weaning and lifetime growth performance (Lawlor et al, 2002). There is a difference between colostrum and lactogenic immunity and how they protect piglets from pathogens they may be exposed to; especially enteric pathogens. Piglet enteric problems in the farrowing house are a major contributor to poor performance. To maximize piglet survival, pigs must obtain sufficient, good quality colostrum in a timely manner.

Colostrum

The Oxford Language dictionary defines colostrum as: "Clear yellowish liquid secreted by the mammary glands of women and female mammals a few months before and a few days after parturition, until the milk rises; it is characterized by being rich in protein and mineral salts, with a low proportion of lactose." Pigs are not able to obtain antibodies from their mothers while in utero due to the placental characteristics. This necessitates piglets obtain all their initial passive antibodies through colostrum. It is estimated that piglets need about 240 - 255 ml (1.5 kg X 160 – 170 ml/kg) of colostrum to survive (Le Dividich et al, 2005). Piglet survival starts to dramatically decrease when colostrum intake is < 200 ml (Ferrari et. al, 2014). These needs are not only for the antibodies (IgG) needed but also for the glucose and fat (both are energy sources) found in colostrum. A recent study by Foisnet et al (2010) estimated the average sow produced 3.22 ± 0.34 kg of colostrum (range 0.85 – 4.80 kg). These are similar ranges found by Devillers et al. (2005) which estimated colostrum production to average 3.6 kg with a range of 1.9 – 5.3 kg. Low colostrum production is not related to litter size or birth weight or due to the inability of newborn piglets to nurse (Foisnet et al, 2010).

Many publications emphasize the importance of allowing piglets to obtain colostrum within the first 24-36 after birth before gut closure occurs. It is true that gut closure occurs, but what is more important is to emphasize that this closure is exponential and therefore from a producer standpoint, making sure that piglets get colostrum within the first 6 hours of life is critical. This can be seen in **Figure 1** above adapted from Miller et al (1962). These changes in gut absorption are due to physiologic changes occurring in the intestine related to protein digestion as well as physical changes in the intestine cells as well (tightening of junctions between cells). In a study by Foisnet et al (2010) it was found that the average time between birth and the first suckle (colostrum) was 29 ± 2 min.

Colostrum yield and mean piglet birth weight are important determinants of newborn viability. Birth order also plays an important role in determining

which piglets get access to the most colostrum as reported in the review article by Farmer and Quesnel (2009). This same article emphasizes that research supports the theory that it is the sow which limits the quantity of colostrum pigs can consume in a day. The overall mortality rate of piglets within the first two days of life is significantly different between litters nursing off low-colostrum producing sows than in litters with high-producing sows (21 ± 10 vs. 4 ± 3%. *P*=0.04) (Foisnet et al, 2010).

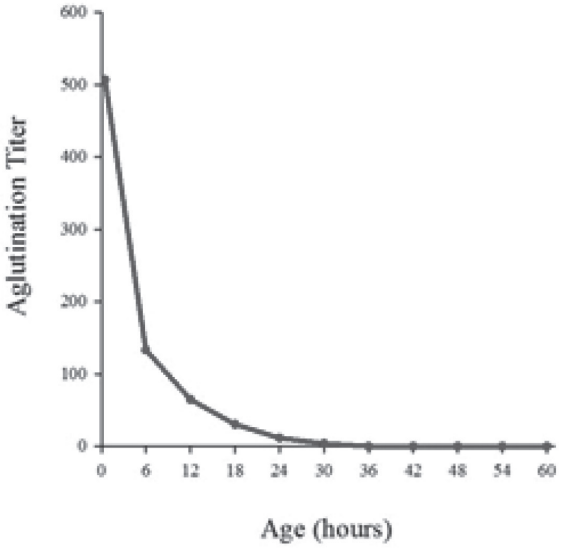

Figure 1. Serum antibody titer in piglets absorbing antibodies from colostrum.

Colostrum also plays an important role in eliciting dramatic changes in intestinal growth, structure and function of newborn pigs during the first 6 hours of suckling. This is highly related to the amount of colostrum ingested and can result in approximately 100-fold increase in absorptive area in the intestines (review by Farmer et al, 2006). It should be the goal of all farrowing house personnel to maximize piglet immunity and intestinal function by maximizing the opportunity for piglets to have access to good amounts of high quality colostrum as soon as possible after birth. This requires not only that the mothers produce the colostrum, but that the right husbandry skills are used to enable this process.

Lactogenic Immunity

After the introduction of porcine epidemic diarrhea virus in the U.S. we quickly were reminded that piglet immunity was not only related to colostrum or IgG but also to IgA. IgA molecules contain two immunoglobulins domains linked together. Secretory IgA (sIgA) includes this IgA dimer with a secretory component attached. It is in the form of sIgA that immunoglobulins are secreted into mucosal surfaces. The secretory component serves to protect the IgA from acids and digestion allowing it to serve as mucosal immunity. Remember that immunoglobulins are proteins and if they were not protected, they would be digested just like other proteins and would not be able to function as part of the immune system.

The sIgA is also secreted by sows into their milk. sIgA from either sow's

milk or produced by the piglet, help block pathogens at the luminal surface of the intestine, preventing them from being able to bind to receptors on intestinal epithelium and thus preventing them from causing disease. This is true for bacteria, virus, toxins, or other pathogens.

The concentration of the different immunoglobulins in milk changes over lactation. Initially it is heavy with IgG (colostrum) and then quickly changes to primarily sIgA as seen on **Table 1**.

It is important to not that although percentage wise there are some dramatic changes in percentage of immunoglobulin types, the actual concentration of IgA is slightly higher in colostrum but because IgG is so highly concentrated in colostrum, the overall percentage of IgA is low initially.

For diseases like PED, there is need for the lactogenic immunity to be working at the mucosal surface to prevent the attachment, and thus infection of intestinal epithelial. IgG would be helpful if the pathogen were to go systemic, but mucosal immunity is critical when the pathogen needs to be blocked at the mucosal surface. This became very apparent during PED outbreaks. Piglets that were weaned, but kept in the farrowing house, would immediately break with severe diarrhea while other piglets that were nursing sows were initially protected. There is the continuous need for sIgA in milk to be "coating" the intestinal lumen with protective immunoglobulins.

Table 1. Change in percentage of immunoglobulins in sows milk over lactation period. Adapted from Markowska-Daniel and Pomorska-Mól (2010).

	IgG	IgM	IgA
1 h	75%	7%	18%
6 h	76%	6%	18%
12 h	77%	6%	17%
24 h	59%	13%	28%
6 d	30%	20%	50%
12 d	20%	21%	59%
18 d	19%	24%	57%
28 d	17%	21%	62%

Husbandry

1. Minimizing pathogen exposure

Disease does not occur unless there are three conditions that are met. You must first have a pathogen that is viable and in high enough numbers to cause disease. Then you need to get these pathogens in contact with the pig. Finally you need to have a pig that is susceptible to the pathogen and therefore disease can manifest. One of the first things to do is to eliminate, if not minimize, pathogen exposure. There are several ways this can be achieved. In the case of enteric problems, other than TGE, most of the other pathogens we deal with are commonly found in farms (*Clostridia*, *E. coli*, Rotaviruses, PED, and Coccidia). Three of the most common practices to reduce pathogen exposure to the newborn piglets involve the cleaning and disinfecting of the farrowing crate, cleaning of the sow before moving into farrowing rooms, and scraping manure behind the sows. These practices make sense and most are supported by some research.

Washing, when done correctly, will remove >99.99% of the microorganisms in the environment. This can be done in conjunction with detergents and hot water to maximize the efficiency and effectiveness of this process. Then the right disinfectant needs to be used targeting specific pathogens on the farm. The disinfectant serves just as the added bonus and should not be relied as the primary means of pathogen control. This is because most disinfectant are inactivated by organic matter and therefore will not be effective unless all organic debris is first removed from the farrowing house. The effect of poor hygiene in morbidity and mortality associated with enteric disease was demonstrated by Svendsen et al (1975).

Washing the sow before moving into the farrowing will minimize the chances of brining in extra manure from the gestation barn. This is probably more important in outdoor facilities, but even in todays confined environment, some sows get pretty dirty. Cleaning the sow especially regarding the udder and the vulvar area will minimize pathogen exposure especially considering these animals are being placed in a nice clean farrrowing crate. It is also a psychological process that helps emphasize the importance of cleanliness. Finally scraping farrrowing crates is not a fun job, but can be an important one. I am not familiar with any research to support the practice, but it just makes common sense that the less manure there is in the back of the crate when baby pigs are born, the less likely they will be exposed to high numbers of different pathogens. Remember that these newborn pigs also have an umbilical cord that has a fresh open wound and will be dragged around right after birth.

Field data also supports the concept of pathogen load. Those piglets that are born first in a room will take 3 – 4 days before they will start scouring while

those born later in the week will start scouring in 24 hours (Cutler et al, 2006). Environmental pathogen buildup can occur quite rapidly especially during an outbreak with enteric pathogens.

2. Farrowing assistance and immediate post-natal care

Over 50% of pre-weaning mortality occurs within the first 3 days after birth with most piglets dying having had consumed much less colostrum than survivors in the first day of life (review in Foisnet et al, 2010). Additional supervision of piglets in the first 3 days of life has been shown to decrease mortality from 1.29 to 0.85 pigs per litter (Probst Miller, 2007). To maximize piglet care one must be present at the time of farrowing to be able to help these newborn piglets sooner rather than later. In Foisnet et al (2010) it was calculated that the average duration of farrowing for 16 sows used in three replicates was 284 ± 50 min. In a study by Gunvaldsen et al (2007) even with the use of induction protocols, 60% of the sows started farrowing overnight. This same study showed that for every day of gestation, piglet growth increased by 26g ($P< 0.01$). This translated into a pig that averaged 576 g less ($P< 0.01$) at 16 day of age and was 2.0 times more likely to have a relative risk for higher morbidity ($P< 0.01$). The induction of premature farrrowing also affects the composition of colostrum and milk especially in regards to fat (Jackson et al, 1995). Fat is an important energy source needed for newborn piglet survival as pigs are born with minimal fat stores.

3. Split-suckling and cross-fostering

The concept of split-suckling and cross-fostering theoretically make sense, but research does not always support the practices. With split-sucking the idea is to allow the piglets to maximize opportunity for colostrum intake. I have been unable to find research supporting the practice but I think there are many challenges. A key point is that split-suckling does have the potential of working if it is done properly. With most piglets being born overnight, it is hard to know how long the pigs have really been born. This is critical as from the colostrum section we know that the sooner we get pigs to nurse, the better the chances for absorption of antibodies. If not done properly, we can actually create more variation in the process.

A study by Donovan and Dritz (2000) showed there were no statistical difference between split-suckled groups in ADG, weaning weights, and serum IgG concentrations. They did find that the percentage of pigs weighing < 3.6 kg at weaning was higher in the control group (1.3 and 1.6% vs. 3%, $P \leq 0.05$). In this study they split suckled for 2 hours within the first 24 hours of life. It is difficult to know what the effects of just split suckling in the first 6 hours of

life could have on the piglets.

In regards to cross-foster (moving pigs from one sow to another) the overwhelming data suggest that although litter weight variation is reduced, individual pig performance is actually compromised (Straw, 1997, Cutler et al, 2006). Price et al (1994) reported that in pigs over 2 days old < 50% of pigs had suckled 6 hours after being moved to a new dam. Pieters and Bandrick (2008) showed that cross-fostering can help transfer antibodies as long as it occurs within the first 6 hours after initial colostrum intake (**Table 2**).

Dewey et al (2008) have also shown that cross-fostering before and after 1 day of life can have a negative impact on piglet weight at 16 days of age. In their multivariate model, after controlling for other significant parameters, piglets cross-fostered before day 1 were 0.18 kg smaller (P=0.002) and those cross-fostered after day 2 were 0.80 kg smaller (P=0.0001) at 16 days of age than those not fostered. Wattanaphansak et al (2002) also have shown that continuous cross-fostering created almost 3 times as many light weight pigs at weaning than non-cross-fostered litters. They speculated that this could have been due to aggressive fighting amongst comingled littermates. This aggressive fighting could result in less milk consumption by these piglets.

Table 2. Proportion of piglets positive to *Mycoplasma hyopneumoniae antibodies* (ELISA). Adapted from Pieters and Bandrick (2008).

	Hours nursing before cross-fostering				Not cross-fostered
Group	0	6	12	20	
Vax Control	NA	NA	NA	NA	10/10 (100%)
Unvax Control	NA	NA	NA	NA	0/26 (0%)
Vax. –> Vax.	12/12 (100%)	11/11 (100%)	11/11 (100%)	10/10 (100%)	11/11 (100%)
Vax. –> UnVax.	0/10 (0%)	10/10 (100%)	10/10 (100%)	9/9 (100%)	9/9 (100%)
UnVax. –> Vax.	10/10 (100%)	7/9 (78%)	1/10 (10%)	0/8 (0%)	0/8 (0%)

4. Chilling

A brief note is important in making sure that the environment which these newborn piglets are raised is adequate. It is critical to remember that a clean, warm and dry environment is desirable. The challenge becomes in establishing room temperatures and zonal heating in order to maximize sow feed intake, which has a direct impact on lactation, and still meet piglet needs. Newborn piglets has a lower critical temperature (LTC) range of about 30 – 34°C while sows have a LCT around 15 – 19°C (review in Cutler et al, 2006). For the first 2 days of life, piglets have difficulty dealing with cold stress (temp < 34°C) due to physiological immaturity which does not allow them to mobilize carbohydrate

energy reserves (glycogen) efficiently (review in Cutler et al, 2006).

From an immune system standpoint, chilled pigs use energy directed to warming up themselves instead of growing and developing their own immune protection (antibody production uses a lot of energy). Intestinal motility is also slowed down at lower temperatures which then predispose piglets to enteric diseases. Decreased intestinal motility will allow for bacterial overgrowth to occur allowing more time and more pathogens to be exposed to the intestinal tract. Intestinal motility serves as part of the body's inmate immune system.

Conclusions

Weaning weight is considered one of the most important factors impacting post-weaning and lifetime growth performance (Lawlor et al, 2002). Piglet enteric diseases are a significant contributor to piglet morbidity and mortality in the farrowing house. Piglets must be care for properly in order to maximize their immunity which will ultimately have a better outcome on their survivability and performance during this early phase of life. Proper colostrum, lactogenic immunity, and husbandry management are critical in helping maximize piglet survival. A better understanding of the mechanism for diarrhea by the most common pathogens found in the pre-weaning period are critical in better diagnosing, treating, and prevention of enteric problems in the herd.

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Post-weaning diarrhea and its relation with the piglet's intestinal health

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Introduction - Post-weaning diarrhea and intestinal health

Post-weaning diarrhea is one of the most critical conditions in modern pig farming. The disease occurs in the two weeks following weaning, occurring with severe diarrhea that leads to dehydration and death [1]. In Brazil, the diarrhea is present in most farms [2].

It is known that the disease is a result of numerous factors that lead to loss of intestinal function. Weaning piglets have villous atrophy and crypts hypertrophy and increased epithelial cell mitosis. Changes occur in the production rate of new enterocytes in the crypt, initially with a reduced formation rate of new enterocytes, and then with a compensatory increase for epithelial replacement. The largest changes in the intestinal architecture occur around five days after weaning, recovering within six days. In this period when the intestinal architecture is impaired, there is also less production of enzymes being secreted in the epithelial microvilli. There is less production of lactase, amino-peptidase, and maltase, for example.

2. Causal and predisposing factors for post-weaning diarrhea

"Intestinal health" is a broad concept, which depends on numerous factors[3]. Therefore, post-weaning diarrhea is a multicausal disease. There is not a single predisposing agent or even a microorganism that can individually lead to the onset of the disease[20]. The individual analysis of each one of these factors often leads to different conclusions from what is seeing in an integrated analysis. It is the integrated analysis or risk analysis that promotes well-done risk management to mitigate the triggering factors. The removal of causal factors is synonymous with prevention. Even when it is not possible to eliminate the disease, the risk analysis allows a clear understanding of the problem for eventual treatments.

For the risk analysis, it is necessary to use practical and measurable criteria that help in diagnosing the situation and correcting errors. There are

many concepts from adventitious theories that can even easily explain where the technicians' commitment should be. As the well-known adage of the economic theory states: "You can't manage what you don't measure, you don't measure what you don't define, you don't define what you don't understand and there is no success in what you don't manage". So, in a practical and applied way five criteria are usually used to define intestinal health. These are points on which we can intervene to prevent post-weaning diarrhea:

1- Effective digestion and absorption of nutrients
2- Absence of gastrointestinal infections
3- Normal and stable microbiota
4- Effective immune status
5- Good conditions of well-being

Figure 1 shows the combination of these causal factors for post-weaning diarrhea in a common condition. These factors will be detailed below.

2.1. Management and environmental factors

The management of weaning is evidently affecting the occurrence of diarrhea. The swine management and the environment influence all the five factors mentioned above.

Inappropriate management influences the occurrence of infections, the imbalance of the microbiota, and well-being. The lack of a sanitary break is the measure with a higher impact on the occurrence of post-weaning diarrhea. Although it is a primary recommendation in animal husbandry, in some regions, up to 80% of the farms do not practice a sanitary break in the nursery. Then, the high animal density in the nursery comes as one of the most relevant factors for diarrhea, as it generates stress and interferes from immunity to nutrition[2].

Management also directly influences immunity: early weaning causes disease because it does not allow the piglet to ingest milk IgA (post-colostrum). Thus, in the European Union, for example, weaning is recommended at least 28 days old.

In another example, management influences good nutrition: As can be seen in **Figure 1**, the low feed consumption in post-weaning, or feed intake rate (FIR), can be considered a significant trigger for breaking intestinal homeostasis. Piglets reduce feed intake at weaning, mainly due to changes in the physical form of the feed - from liquid to solid - and due to stress, common in this situation. Low consumption compromises luminal nutrition, generate stress factors, and compromises the structure and function of the intestine, inducing damage. The vile-crypt architecture becomes extremally compromised, at the same time impairing the intestinal barrier function and finally altering the intestinal microbiota with dysbiosis. Dysbiosis will be further explored later. Half of the weaned piglets consume feed for the

first time 24 hours after weaning. One-tenth still do not eat until 48 hours after weaning. The energy requirements under these conditions often only reach the necessary level 3 to 4 days after weaning. Eventually, under very inadequate management conditions, the level of pre-weaning energy intake will only be reached 8 to 14 days after weaning. In these situations, it is difficult to imagine a strategy with later products or processes that can minimize the damage caused. There are many consequences of these nutritional imbalances throughout the intestine, from the morphological, functional, immune, and microbiological aspects.

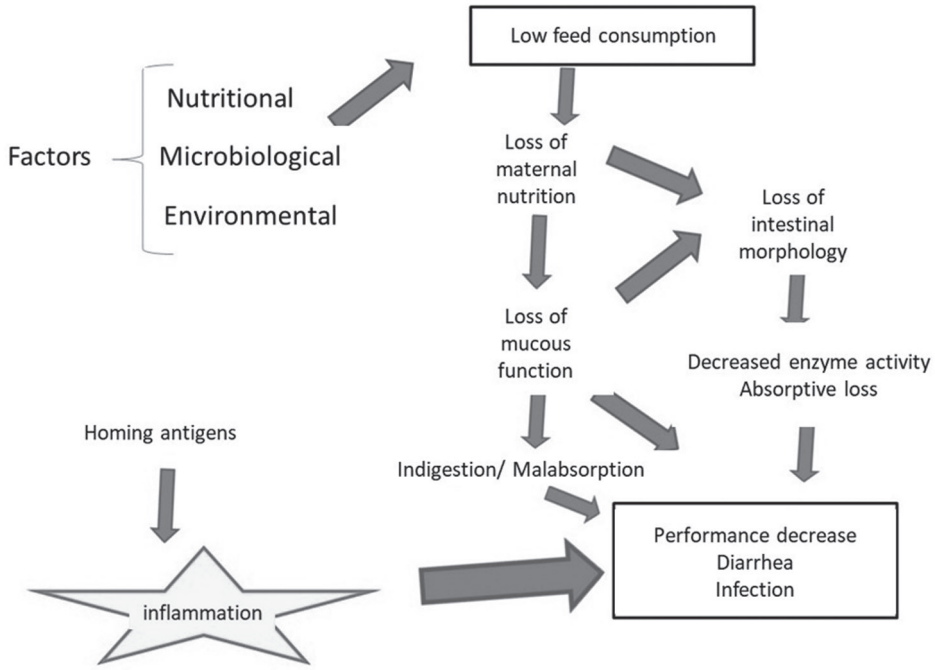

Figure 1. Causal connections of post-weaning diarrhea.

In these conditions, two days after weaning, the jejunum decreases its electrical resistance, which is associated with several functional damages to the enterocyte. For example, glucose absorption may remain impaired until two weeks after weaning. The enterocyte reduces the use of nutrients and loses its adhesion to the surrounding cells. In this context, the intestine will be more permeable to pathogens after weaning[21].

Injuries, or sometimes just the delayed development caused by post-weaning fasting, have lasting consequences. When the intestinal epithelium is damaged by fasting, "translocation" occurs, a more numerous passages of

bacteria, viruses, and toxins through the intestinal wall, reaching other organs. Upon reaching the lamina propria, they trigger more inflammatory reactions; it must be remembered that inflammation can reduce food consumption due to the impact it has on the hypothalamus. Thus, a vicious cycle is established in which fasting permeates the intestine and causes more inflammation, which prolongs fasting.

Stress is a management factor that can aggravate the condition induced by the fasting period. Heat stress, for example, exacerbates bacterial diarrhea in pigs. It is widely known that stress can influence immune function through the hormone cortisol. Besides, enterocytes are directly affected, as well. These cells lose their absorptive capacity in response to the Corticotrophin Releasing Factor[4]. Thus, farms with effective temperature control have a lower incidence of diarrhea, for example [5].

Thus, weaning *per se* and the anorexia generated are conditions that cause inflammation and even without specific injuries are already causes of this post-weaning diarrhea. In this case, just stimulating or making consumption possible some conditions improve. However, in these conditions, infectious challenges can become difficult to control, depending on the microorganisms involved. As mentioned above, the intestine loses its absorptive capacity due to the change in diet and the stress of weaning.

2.2. Microbial factors - provocative agents

Escherichia coli is the most common microbial agent found in post-weaning diarrhea. In addition to its prevalence, its importance has been increasing due to the occurrence of multidrug resistance to antibiotics [6]. Some virulence factors are significant for the occurrence of diarrhea, and that is why *E. coli* Enterotoxigenc (ETEC) and Enteropathogenic (EPEC) are the most found in these cases [1]. The most common ETEC in post-weaning diarrhea has fimbriae F4 (K88) or F18, with LPS from various serogroups, such as O8, O157, and O149. The fimbria F4 mediates the connection with a specific intestinal receptor (F4R) - that receptor is not always present in the porcine intestine, and therefore there is a genetic component for the onset of diarrhea.

E. coli leads to diarrhea through the production of heat-stable (ST) or heat-labile (LT) toxins causing the loss of electrolytes by the enterocytes. The ability to produce these toxins is the reason for their classification as Enterotoxigenic. The production of toxins leads to water leaving the intestinal lumen and, therefore, to diarrhea.

In Brazil, toxins are present in at least 50% of *E. coli* isolates from neonatal diarrhea. Both LT and ST toxins are present, and in general, are accompanied by the presence of fimbriae in bacteria. The fimbriae F18, F4, and F5 are the most common, although there is significant variation in the presence of these virulence factors between the regions of the country[7–9]. Both fimbriae

and flagella assist in bacterial adhesion to the intestinal surface (**Figure 2**). National strains are also highly resistant to antibiotics. In several analyzes, strains resistant to more than 3 antimicrobials were found simultaneously, reflecting the need to use other approaches to control pathogens [10].

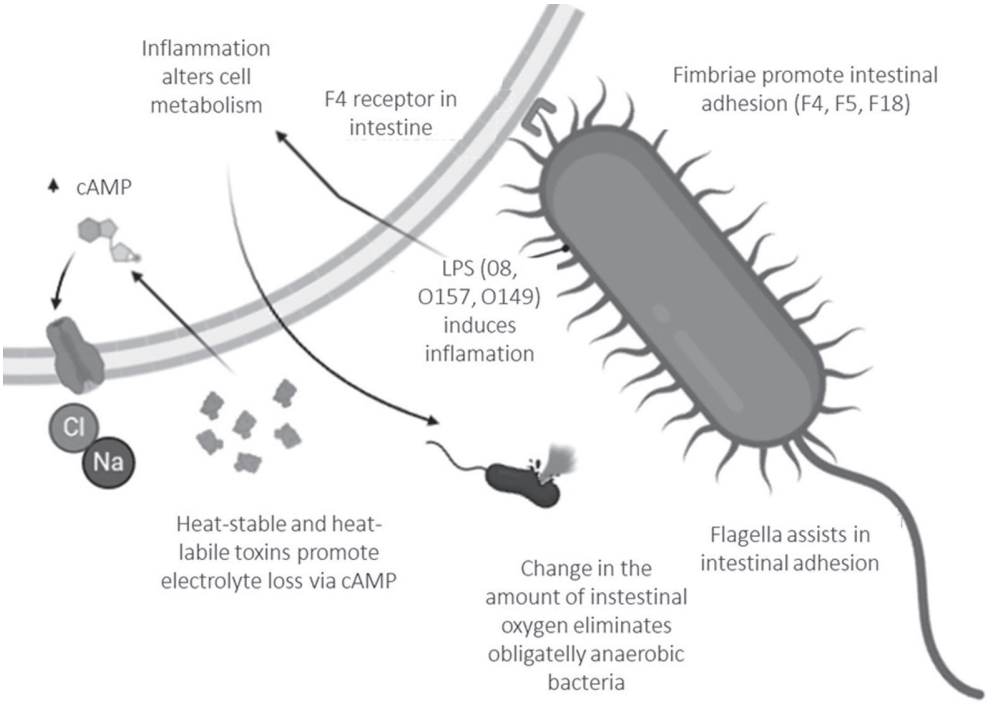

Figure 2. Pathogenic mechanisms of *E. coli* in the porcine intestine in post-weaning diarrhea.

However, it must be reiterated that post-weaning diarrhea is a multifactorial disease. Other infectious agents, such as rotaviruses, can act simultaneously in diarrhea, aggravating the clinical picture [11]. These viruses are capable of producing proteins similar to bacterial toxins, which intensifies the condition in cases of co-infections. Besides, rotaviruses directly infect the intestinal epithelium, causing villous flattening, again worsening diarrhea caused by *E. coli*.

2.3. Microbial factors - Intestinal microbiota

It is possible to define microbiota as the bacterial population present in an organ. The microbiota impacts the occurrence of diarrhea due to its "permissiveness" for the proliferation of *E. coli*. Considering the name *Enterobacteriaceae*, the family that comprises the *E. coli*, one can have

the impression that these bacteria are predominate in the enteric system. However, these bacteria are only a small fraction of the microbiota, often making up less than 1% of the intestinal bacteria. Most intestinal bacteria are obligate anaerobes, so they are little studied in the laboratory. **Table 1** shows the bacterial genus that are always present in the pig GI microbiota [12]. Suckling is one of the most relevant factors in the composition of piglet microbiota. Each sow has the potential to induce a healthier or less healthy microbiota in its piglets. Also, suckling promotes a microbiota focused on the milk diet to which piglets are submitted. Thus, in the weaning transition, the microbiota needs to be adapted quickly to the change in diet [13]. Therefore, understanding the microbiota is essential to understand the role of one of the most important bacteria in post-weaning diarrhea, *E. coli*.

As previously discussed, the process that leads to diarrhea can pass through a phase of anorexia after weaning. Anorexia can occur due to the stress of weaning, the low frequency of feeding, or the lack of space in the feeders, for example. This period leads to disturbances in the constitution of the microbiota, which opens space for *E. coli*. Initially, disorders caused by anorexia impair intestinal structure, as discussed above. Thus, a large part of the nutrients passes without digestion to the final portions of the intestine, favoring saccharolytic bacteria, such as *E. coli*. Fasting is also harmful to enterocytes, which can cause local inflammation. The death of enterocytes during fasting is a possible inflammatory activator. Inflammation, in turn, increases oxygenation in the intestine - one of the central points of inflammation is the increase in diameter and vascular permeability, that is, greater local perfusion. Since most intestinal bacteria are anaerobic, increased oxygenation is harmful to a healthy microbiota. Enterobacteriaceae, on the other hand, are facultative anaerobic bacteria and can grow in an atmosphere with oxygen. Thus, during inflammation, *E. coli* proliferate without competition from other bacteria. Furthermore, during weaning, piglets produce less alkaline phosphatase, an enzyme essential in the breakdown of bacterial LPS, which aggravates intestinal inflammation [14].

The inflammatory response in the intestine also produces reactive species such as NO (nitric oxide), which has antimicrobial properties. However, the NO released in the intestinal lumen is quickly transformed into nitrate. The solid nitrate environment gives competitive advantages to *E. coli*, which has genes for the production of nitrate reductase, absent in clostridia and bacteroidetes. This situation is frequent 7 days after weaning, occurring simultaneously with the abundance of oxygen and blood flow favoring facultative aerobics such as enterobacteria, with the reduction of obligately anaerobic and a decrease in diversity.

3. Interventions

Many interesting articles and books were already written about post-weaning diarrhea, some of which are mentioned here and can be consulted by anyone who wishes to delve deeper into the use of drugs for an intervention on the disease. In this section, we intend to present the effects of interventions against diarrhea from a different point of view: that of the intestinal microbiota. It is expected to demonstrate that some interventions against diarrhea, always evaluated for their short-term benefits, have lasting consequences on animals in the form of changes in the microbiota.

Table 1. Composition of the microbiota of animals in different situations 13,15–19. "↑", a relative increase in the bacterial group in question. In the "weaning" columns, the comparison is made with healthy pre-weaning piglets. In the "treatment" columns, the comparison is made for animals with post-weaning diarrhea that have not received treatment. "↓", reduction of the bacteria group. "-", with no change in the bacteria group.

Bacterial genus or microbiota condition	Semple type	Healthy animals	Diarrhea	Weaning (at beginning)	One month after weaning	Antibiotics	Treating with essential oils	Treating with probiotics
Prevotella	Feces or GI content*	Always**	↑/↓	↑/-	↑	↓	↓	↓
Clostridium	Feces or GI content*	Always	↓	↑		↑		↑
Alloprevotella	Feces	Always			↑	↓		↓
Ruminococcus	Feces or GI content*	Always	↓		↑	↓	↑	↓
Blautia	GI content*	Always		↑				↑
Lactobacillus	GI content*	Alwayse	↓	↓	↑		↑	↑
E. coli e outras *Enterobacteriaceae*	Variable	Variable		↑/-	↓	↑	↓	↓
Streptococcus	Variable	Variable	↓	↓		↑/↓	↑	↓
Faecalibacterium	Variable	Variable				↓		↑
Bacterial Diversity	Variable	-	↓	↓/-	↑	-		

* These bacteria are present in samples from all parts of the gastrointestinal tract.

** These bacteria are always present at some part of gastrointestinal tract of healthy pigs.

Since the balance between health and intestinal disease will depend on the constitution of a healthy microbiota, the consequences of some agents on the intestinal bacterial population are shown in **Table 1**. This information comes from studies on the microbiome. The microbiome is a mirror of the microbiota, it is the genetic makeup of microorganisms in an organ's microbiota.

4. Conclusion

The data presented in this chapter denote to professionals the need to connect so much knowledge acquired from different disciplines and prove why the ability to gather concepts determines the quality of the professional and the success of the measures implemented. When looking for strategies to minimize the impact of post-weaning diarrhea, the importance of knowing the pathogenesis of the bacteria, in particular, becomes evident, mainly when associated with certain viruses. At the same time, understanding the digestive physiology and management inherent in this early phase shows how and why management associated with nutrition and ambiance is a determining factor in preventing these episodes or in mitigating them. The subject is complex, and for this reason, it should be the object of study and daily application in the routine. Only then, measures beyond those that are common to all production systems can be customized within a reality with so many additive alternatives other than the habitual use of antibiotics.

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Economic impact of the enteric diseases

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Introduction

Understanding how much enteric disease costs helps producers make rational and informed decisions about how much time, money, and other resources should be devoted to reducing the impact of disease. In short, a cost-benefit analysis of interventions to reduce disease can't be done well unless some knowledge of the costs and benefits can be found. If it is so important to have knowledge of what disease costs, why is there not more information available? There are several reasons. It is difficult to get enough good data to make reasonable estimates. When good data can be obtained, attributing differences or changes in the data to a specific disease or diseases is always a challenge. Another challenge is the lack of researchers with the necessary knowledge of production, disease, and economics to turn the data into reasonable estimates.

This paper presents results from two separate studies on the cost of swine enteric diseases. The first is a study of the economic losses caused by major diseases, not just enteric diseases, in the United States from a survey of veterinarians or producers[1]. It is an old study, but results still provide some context for today. The second is a study of the economic losses caused by ileitis based on data from published observational and experimental studies comparing challenged and unchallenged controls.

Economic losses caused by enteric disease from a survey of veterinarians

Materials and methods

The companies producing more than 150,000 pigs per year were identified as the population of interest. This population was further segmented by size, vertical integration, and geographic location in order to assure representation of the entire population of interest. Companies with 7,500 to 25,000 sows were considered "medium" sized while companies with more than 25,000 sows were considered "large." Only companies with more than 25,000 sows were segmented as integrated or not integrated since very few producers with fewer

than 25,000 sows were vertically integrated. The United States was divided into three geographic regions based upon USDA's farm production regions as follows: East (Appalachian, Delta, Northeast, Southeast), Midwest (Lake States, Corn Belt), and West (Northern Plains, Pacific Northwest, Southern Plains, Mountain).

Two production companies from each of the nine segments that resulted were selected for inclusion in the study. A third medium-sized company in the Midwest was surveyed for a total of 19 companies included in the study. The selection of companies was based on the anticipated willingness to participate and, therefore, not random. Only one company asked to participate declined. A survey was developed and administered to a single veterinarian at each company through face-to-face, personal interviews. The same interviewer, also a swine veterinarian, administered all of the surveys in a consistent manner. All of the surveys were conducted between November of 2005 and February of 2006.

Veterinarians were asked to identify the major health challenges, which consisted of individual pathogens or diseases or combinations. For the major health challenges identified, they were asked to estimate the animal health costs and productivity losses in affected herds as well as the percentage of animals in affected herds. A production and economic model was used to place a value on the estimated lost productivity and animal health costs for each of the major health challenges.

Results

The enteric diseases identified as major health challenges, by the phase of production are reported in **Table 1**. The estimated economic losses in herds affected by each enteric health challenge and the percentage of animals in affected herds are also reported in **Table 1**.

Table 1. Estimated economic loss in affected herds and the percentage of animals in affected herds for major enteric health challenges.

	Economic loss in affected herds (US$/pig marketed)			Percentage of animals in affected herds		
Health Challenge	**Breeding**	**Nursery**	**Finishing**	**Breeding**	**Nursery**	**Finishing**
Clostridium perfringens Type A	$1.78			21.6%		
Ileitis (*Lawsonia intracellularis*)	$0.44		$4.65	8.7%		10.6%
Rotavirus + *E.coli* (farrowing)	$2.16			14.8%		
Rotavirus	$1.59			19.0%	5.4%	
Gastric ulcers			$3.20	1.3%		8.6%
Clostridium perfringens Type C	$3.50			3.2%		
Coccidiosis	$0.62	$0.54		16.7%	2.7%	
Clostridium difficile	$0.61			16.4%		
E.coli (post weaning)		$1.62			3.9%	0.6%
E. coli (farrowing)	$0.61			3.6%		
Hemorrhagic bowel syndrome			$3.72			0.4%

Economic losses caused by ileitis using data from the literature

Materials and methods

Published studies provide a basis for estimating the impact of ileitis on finishing ADG, FCR and mortality. Several experimental challenge studies, comparing non-challenged (negative control) pigs to challenged (positive control) pigs, were found with a review of the literature (Shurson et al., 2002; Beckler et al., 2012; Collins et al., 2014). All of the studies included a negative control, and at least one group of pigs challenged with *Lawsonia intracellularis*. None of the studies included any groups of pigs treated with a vaccine or antimicrobial. The age of pigs when challenged and the challenge dose varied in each study. The age of pigs in all of the studies cited above was greater than 42 days of age. In addition, a case-control study comparing herds affected by ileitis to those not affected by the disease, determined by their serological status, was also identified (Fourchon et. Al., 2000).

For the purpose of estimating the economic losses due to ileitis, three scenarios were developed: 1) Unaffected by ileitis, 2) Affected by ileitis using the lower bound of estimated production impacts from the case-control and experimental challenge studies and 3) Affected by ileitis using the upper bound of estimated production impacts from the case-control and experimental challenge studies. A production and economic model was used to calculate the value of the production losses for the second and third scenarios relative scenario 1.

Results

The production losses for each scenario are summarized in **Figure 1**. Differences between the two affected scenarios and the unaffected scenario represent estimated productivity losses due to ileitis. The results of the economic analysis are presented in **Table 2**. The value of the lost productivity caused by ileitis ranged from US$5.98 for the lower bound to US$16.94 for the upper bound.

Figure 1. Finishing average daily gain, feed conversion and mortality for each scenario

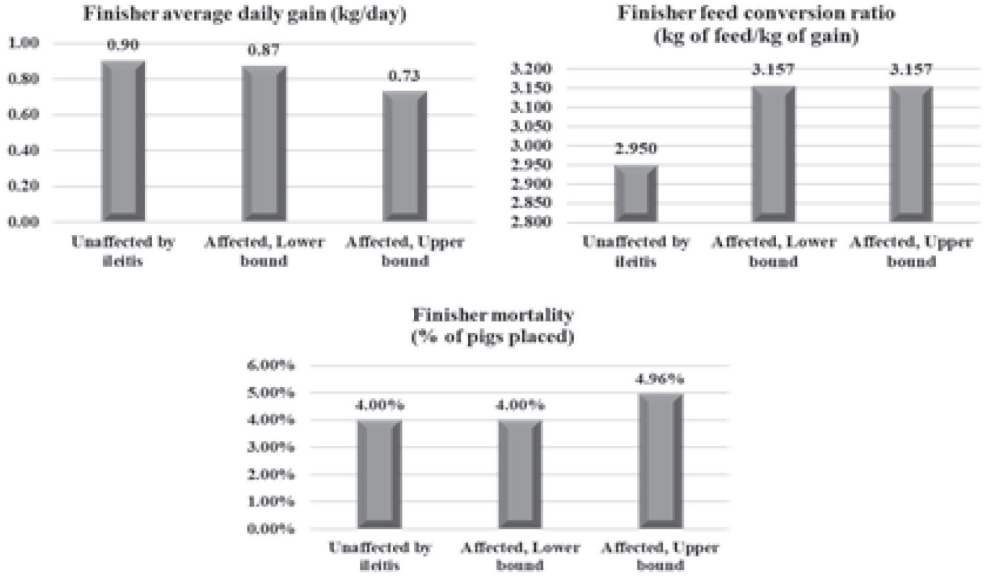

Table 2. Estimated value of poorer ADG, FCR, and mortality caused by ileitis.

	Unaffected by ileitis	Affected, Lower bound[1]	Change from Unaffected	Affected, Upper bound[2]	Change from Unaffected
Net profit ($/pig marketed)	US$18,84	US$12,86	-US$5,98	US$1,90	-US$16,94

[1]Lower bound:

- ADG decreases from 0.90 to 0.87 kg/day (-3.0%)
- FCR increases from 2.950 to 3.157 kg feed/kg gain (+7.0%)

Mortality rate was unchanged

[2]Upper bound:

- ADG decreases from 0.90 to 0.73 kg/day (-19.0%)
- FCR increases from 2.950 to 3.157 kg feed/kg gain (+7.0%)
- Mortality increased from 4.0% to 5.0% (+24%)

Conclusions

Both studies demonstrate the economic losses from swine enteric disease are very significant. The results from the survey of veterinarians provide estimates of the economic impact of swine enteric diseases that were causing the most significant losses in 2005 and 2006 when the survey was conducted. The reported losses include the value of lost productivity and money spent on animal health costs, including vaccines, pharmaceuticals, diagnostics, and veterinary services. The estimated economic losses caused by ileitis using data

from the literature represent only the value of productivity losses. Since no studies with vaccines or antimicrobials were included, the values represent uncontrolled ileitis. The results also represent a very controlled challenge with *Lawsonia intracellularis* and thus do not reflect the infection dynamics found in the field. Therefore the estimated impacts based on the challenge studies may overstate the productivity losses observed in commercial settings.

Acknowledgments

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03

African Swine Fever (ASF)

African Swine Fever: it's Impact and How to Prevent the Introduction Of this Disease into the Herd

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Introduction: ASF in the Southwest Asia and the Philippines

ASF is the biggest crisis to affect livestock in the 20th century. From 2016 to 2020 June 18, submissions to the OIE through the Early Warning System of various countries worldwide peg the losses at 8.2 million heads. Asia accounts for 82% (6.7 million heads) of the total global reported losses [1] – a grave misrepresentation, if stories of under-reporting of numbers by various countries are to be believed.

Worldwide, it has a huge impact on the global economy mainly in terms of feed ingredient, pork, and pork product trade. It threatens the food security of a nation as well, especially for countries with already limited meat production.

With the spread of ASF in Southeast Asia, it is almost a certainty that this disease will eventually reach all pig-producing countries within a few decades. It would be prudent for ASF-free countries to learn from the experiences of countries that were hit and are currently battling ASF. The Philippines is completely surrounded by water, a great epidemiological advantage. Sadly, it wasn't enough to repel ASF. The Philippines is composed of 3 island groups, each with decreasing sow levels as you go south, where the population is predominantly Muslim. The most densely populated area is Metro Manila, which explains why almost 50% of the country's total pork production can be found in the surrounding provinces which can be found in Central Luzon. As of this writing, ASF is present in 26 provinces, all situated in the island groups of Luzon and Mindanao which comprise almost a third of the total number of provinces. The Philippines has been battling ASF for a year, and the disease is still spreading, having recently affected a province in south Luzon.

How ASF started and is spreading

After the People's Republic of China made the initial report in 2018

August, 7 out of 11 countries of the South east Asian Region started reporting in rapid succession in 2019: Vietnam in February, Cambodia in March, Laos in June, The Philippines in July, Myanmar in August, Timor-Leste in September, and Indonesia in November. The other Asian countries that reported were Mongolia in 2019 January, Hong Kong in 2019 May, North Korea in 2019 May, South Korea in 2019 September, Papua New Guinea in 2020 March, and India in 2020 May[1].

The virus isolated from the Philippines is 97-100% compatible with strains from Georgia, Russia, Estonia, Poland, Belgium, china and Vietnam. Based on the partial sequence of the p72 gene, it is a genotype 2 asf virus[2]. Asf entered ph most likely via smuggled pork and pork products from China [3]. Smuggling has been a perennial problem in the Philippines, with some confiscated shipments reaching values of USD 70,000 that tested positive for ASF [4]. The shipments come in by way of the Port of Manila, where they are marketed in metro manila and neighboring provinces. The first 4 outbreaks were reported in 4 provinces within 83 km of the Port of Manila, over a span of 2 months. It's interesting to note that one of those provinces was Metro Manila, the most urbanized province in the country which reported a total of 16 outbreaks. It is quite common in urbanized provinces like Metro Manila to have cart-pushing, ambulant kitchen waste collectors that cater to backyard raisers; one can just imagine the backyard-level augmentation of the viral load in this segment of the pork supply chain (backyard raisers and consumers).

Following the first report in Rizal Province on 2019 July, the next to report was from Bulacan Province in 2019 August. A hog buyer was able to bring ASF-infected hogs to a stockyard where it spread to nearby farms. It quickly spread to its neighboring province Pampanga, which reported on 2019 September, with other nearby provinces in Luzon following within months. A peculiar occurrence is the reporting of ASF in Davao City and Davao del Sur which are situated in the southernmost island group of Mindanao, Davao city being almost a thousand kilometers from Manila.

Once the commercial farms were involved, a greater scale of commercial-level augmentation of the viral load was possible, involving not just small meat stalls but also on a larger scale wet markets, grocery meat shops, and meat processors. An insight into the possible gravity of augmentation in this segment of the pork supply chain would be the fact that ASF has been detected in processed food [5].

At the commercial farm level, one study [6] by M.C. San Esteban, et al. was conducted to assess the possible pathways of transmission. Eleven risk factors were identified in 35 ASF (+) farms in Bulacan and tallied. The top 4 results are as follows: 83% of the farms had external buyer's hog trucks that were positive to ASF detection, 46% were in 500m proximity with other farms, 28% had questionable personnel biosecurity, and 17% had other animals like cats, dogs, & chickens living on the farm. Onze (11%) were prone to flooding, 11%

were situated in areas where a mortality collection truck periodically visited, and 3% were exposed to ASF mortalities. This is comparable with a similar study of A. Kittawornrat[7] on biosecurity in large commercial pig farms in Thailand showing that the biggest risk of ASF contamination comes from the hog selling process, followed by personnel biosecurity and weanling & breeder movement, with pests and feeds posing the lowest risk. Similarly, another study in China [8] shows the greatest risk coming from contaminated vehicles, followed by swill feeding and transport of live pigs and products.

One risk factor apparently not significantly contributing to ASF spread according to the previous risk analysis studies is contaminated drinking water. Dr. Alcrudo, et al mentioned in an ASF detection and diagnosis manual [9] that "Infection via large bodies of water such as lakes and rivers is unlikely because the virus rapidly becomes diluted and will not be present at infective levels". But what about ground water? Is it possible for ASF to be spread this way? Say, you have a mortality pit that is unguarded by a non-permeable membrane. Is it possible for contaminated leachate to reach the underground aquifer and deliver an infective dose to nearby farms? What would be the conditions that will allow this to happen? I imagine it would depend on the distance between the mortality pit to the aquifer, the volume of leachate produced, Soil texture/permeability, and Proximity to other deep wells. Niederwerder's study [10] on the infectious dose of ASF when consumed naturally in liquid or in feed concludes that probability of infection scales exponentially with dose and number of exposures. In the graph on the right, it shows that 10 repeated exposures to a dose of just 1 virus particle delivered via drinking water increases the probability of infection to almost a 100 percent. Another risk factor apparently not making a significant contribution to ASF spread according to the previous risk analysis studies is mechanical transmission by flies. In a study [11] by Fila and Woźniakowski it was concluded that flies like Stomoxys and Tabanus play an important role in the transmission within farms and that research into the identification of new vectors is crucial. An on-going study by R. Parayao on the possibility of the common housefly spreading ASF through mechanical transmission showed RT-PCR and biosensor tests detecting ASF on a pooled sample of legs from 20 flies. It's interesting that the bodies of the flies yielded negative results. It is also interesting to note that comparing the results of RT-PCR and biosensor, there is an indication that the ASF biosensor test, which was developed in the Philippines is more sensitive in detecting ASF. What other risk factors are being overlooked? Much study and research must be done, especially during this time that some farms are trying to repopulate. In my opinion, thoughts of repopulation of a farm should be entertained only when it has been made clear how ASF was introduced in the first place.

To contain the outbreaks, all animals within a 1km radius are culled, animal movement within 7km radius is not permitted, and within a 10km

radius, active surveillance is done. This is according to the ASF contingency plan issued by the Department of Agriculture. The DA also implemented an ASF zoning scheme, aimed at facilitating trade between provinces. This is in response to past events when the local government of most provinces closed down their borders which caused overall disruption of pork supply.

The impact of ASF on consumer demand, hog prices, and swine population

To encourage reporting, indemnity of USD 100 per head is offered to farm owners but only those with 20 pigs or less – essentially backyard raisers. Comparing the lowest price recorded of a 100kg market hog valued at USD100, the indemnity is sufficient to cover the loss. But comparing it with the cost to produce (USD 190) and considering the fact that the indemnity fund will not cover commercial farms, you can imagine most commercial farms doing their best to sell their pigs quickly - whether they have ASF or not. This behaviour of massive selling can cause a chain of events making things worse.

Right now there is disruption of pork supply, but later on it is possible to have a new challenge, this time with a possible oversupply of eggs and chicken meat. The high price of table eggs from 2019 to the present has caused many swine producers to shift to layer production. Existing broiler producers are expanding their operations in response to the pork deficit. The exact numbers are not yet known, but you can imagine what will happen if customer demand in the future does not match this increased production.

Particularly bad news about ASF can drastically lower the demand to pork especially in the wet market, despite a huge effort to convince consumers that ASF is not a public health threat. To counteract this effect, the swine industry with the help of the department of agriculture launched a campaign to make the public less afraid of ASF. It is unfortunate that the effect of the media was too strong, and the demand remained abnormally low until 2020. On a bright note, some meat shop companies report fast recovery of sales in 2019 October and November. This could be due to the perception of consumers that pork sold in meat shops are safer compared with pork sold in wet markets.

The combination of a drastic increase in pork supply in some provinces brought about by movement restrictions and a lingering decrease in demand has a powerful impact on hog prices. The farm gate price has been going down since 2018 July in a smooth, gradual way until 2019 September, when prices dropped drastically. It was during this time that provinces were closing down their borders while farmers were trying to "cash in" before they got hit. You would expect hog prices to go down further during COVID, but this is not the case - an indication of the markedly low status of pork supply [12].

Looking at the sow depopulation in 5 provinces in Central and North Luzon

whose combined initial population was close to half a million sows, from 2019 September to 2020 July there is an estimated 69% sow depopulation in this area, which relates to 16% of the nation's total sow population. According to industry estimates, the Philippines has lost a total of 3.6 million heads (or 380, 837 sow level) [13] through pre-emptive culling (ASF negative farms cashing in), stamping out, and ASF mortality combined.

Preventing entry into the herd

On a national scale, the following control measures are imperative: discovering unknown or identifying underestimated risks, putting a stop to smuggling and swill feeding, increasing awareness of the public without causing panic, sustaining heightened surveillance, improving quarantine capabilities, and strengthening biosecurity throughout the whole pork supply chain. There will be times when harmonious cooperation among different government agencies will not be enough to implement change in a timely fashion; it is during this time when the courage and decisiveness of your country's leaders will be sorely tested.

Summary

The entry and initial spread of ASF is linked to risk factors that are extremely difficult to mitigate, like importation, smuggling and swill feeding. The ASF situation in the Philippines had an immediate negative impact on consumer demand & hog prices and a long-lasting impact on pork supply. Strong political will is critical in preventing and managing an ASF outbreak.

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04 Innovation and Research: abstracts presented in the panel "Respiratory Health"

Risk factors for pleuritis in pigs at slaughter

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Introduction

Adhesions of pleura/pericardium, or just adhesions, are observed at slaughter and are evolutionary consequences of pleuritis/pericarditis that occurred still on the farm, in the finishing phase. According to data analyzed by the SIGSIF (Management Information System of the Federal Inspection Service) regarding the total number of pigs slaughtered in Federal Inspection slaughterhouses in the period from 2012 to 2014, the registered prevalence of this pathology was 4.57%. However, such prevalence is underestimated because the SIF (Federal Inspection Service) records only the predominant pathology and many cases of these adhesions appear at slaughter associated with other more relevant pathologies, for which the carcass is condemned.

The economic importance of these adhesions affects the producer and industry. The impact for the producer is due to the reduction in weight gain, expenses with medicines, and devaluation of the carcasses of slaughtered animals. Each 1% increase in slaughter prevalence increases the slaughter age of the batch by 0.26 days. Also, pigs with pleural adhesions have a carcass weight of approximately 4.4 kg less than individuals from the same batch without the injury. For the industry, the loss assessed in a study carried out in 2016, related to animals slaughtered from 2010 to 2014 in a slaughterhouse located in the southern region of Brazil that exported a large part of the production, the total loss was US$ 1,77/pig slaughtered, mainly due to the export restrictions for affected carcasses.

The main etiologic agents involved in pig serous lesions that are seen at slaughter in the form of pleural/pericardial adhesions are *Pasteurella multocida*, *Actinobacillus hyopneumoniae*, and *Glaesserella parasuis*, however other agents may be less frequently involved, such as *Streptococcus suis*, *Mycoplasma hyorhinis*, and the influenza virus.

To support the review of the inspection legislation regarding pleural adhesions, Embrapa Swine and Poultry carried out a study on 100 affected carcass (50 that had only adherence, without lung injury and 50 that in

addition to adherence also had lung parenchyma injury). In the bacteriological examination of swabs collected from adhesions of the 100 carcasses, it was not possible to isolate any bacteria. This result supported Decree No. 9,013, of March 29, 2017, in § 2 st which states the following: "In cases of pleural adhesions without any type of exudate, resulting from resolved pathological processes and without repercussions in the regional lymphatic chain, the carcass can be released for consumption, after removing the affected areas".

Associated risk factors

In recent years, several scientific studies carried out in other countries have demonstrated many risk factors, with a respective odds ratio (OR), associated with the occurrence of pleural adhesions at slaughter. The most relevant are:

- Management of the herd not allowing downtime: OR = 9.3;
- Maintenance of pigs with an age difference of more than 30 days in the same environment: OR = 6.5;
- Pig movements between pens in growing/finishing phases: OR = 2.2/ *per* movement;
- The partially vs. completely slatted floor at weaning: OR = 21.4;
- Period of downtime in growing/finishing/additional day: OR = 0.86 (preventive effect);
- Presence of *A. pleuropneumoniae* on the farm: OR = 8.8;
- Production type: **wean-to-finish** (OR = 0.10); growth to slaughter (OR = 0.45) - (preventive effects);
- Use of disinfectant between batches in the finishing phase: OR = 0.20 (preventive effect);
- Number of origins in the finishing phase: less than or equal to 3: OR = 0.17 (preventive effect);
- Complete washing between batches: OR = 0.24 (preventive effect);
- A positive association between high levels of dust and ammonia with pleurisy in the total period of growth/finish:
  Dust: OR = 20.9;
  Ammonia: OR = 21.54;
- A positive association between high dust and ammonia levels with pleurisy in the second half;
  Dust: OR = 40,85; P<0,001

In addition to these factors are also mentioned:

- A high density of pig farms in a region;
- Lack or inadequate downtime in the farrowing and late management

practices with piglets such as tail docking, castration...;
- Weaning age below 23 days;
- Room temperature in the growing/finishing barns below the animals' comfort zone.

Conclusion

The prevalence of chronic pleuritis and pericarditis in slaughtered pigs is high, not only in Brazil but in all countries with industrialized pig production. Due to the economic damage caused to producers and the industry by these injuries, it justifies investing more in prevention at the farm level, mainly correcting the existing risk factors. When in a certain slaughterhouse, there is a high prevalence of pleural adherence, the first step is to identify the origin of the animals and establish an etiological diagnosis of the problem. The second step is to take specific control measures (vaccines/treatments according to the identified etiology) and nonspecific (identify and mitigate risk factors).

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Development of oral vaccine for immunization of piglets against *Mycoplasma hyopneumoniae*

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This study aimed to develop an oral vaccine against *Mycoplasma* (*M.*) *hyopneumoniae* by using a nanostructured mesoporous silica (SBA-15) as an adjuvant, and compare its effect with a widely used intramuscular (IM) vaccine (M+PAC, Merck Animal Health, USA). For this purpose, fifty 24 day-old *M. hyopneumoniae*-free piglets were divided into five equal groups for different immunization protocols, consisting of a commercial vaccine (CV) and/or oral immunization (OI). CV piglets received a single dose IM vaccine at 24 days of age (D0); OI piglets received a single dose of oral vaccine at D0; CV+OI piglets received a dose of IM vaccine at D0 and a booster with the oral vaccine 4 weeks later (D28). OI+OI piglets received a dose of the oral vaccine in D0 and a booster with the same vaccine in D28; CONT piglets were the control group, which did not receive any form of immunization. All piglets were challenged with 5 ml of Friis medium containing 10^6 CCU/ml of *M. hyopneumoniae* strain 232 on D49 by tracheal route. Weekly, nasal swabs were collected for IgA measurement (ELISA) and *M. hyopneumoniae* shedding. Fortnightly, serum samples were evaluated for IgG measurement (ELISA). Half of the animals in each group were euthanized 28 days post-infection (D77), and the other half was euthanized 56 days post-infection (D105). At slaughter, lungs of all animals were macroscopically evaluated as the European Pharmacopeia methodology, biological samples such as lung fragments were collected for qPCR and histopathology, and bronchoalveolar fluid (BALF) for qPCR. All immunization protocols showed reduction on Mycoplasma-like macroscopic lung lesions. Primary and memory IgA Ab responses anti-MHYO were

effectively induced by CV and OI vaccines. The use of silica (SBA-15) as an adjuvant for oral immunization of pigs has promising results, which can be an ally in the control of *M. hyopneumoniae* infection.

Why gilts acclimation should be done to control *Mycoplasma hyopneumoniae*?

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Two significant aspects must be considered to understand the reasons why gilts acclimation should be used to control *Mycoplasma (M.) hyopneumoniae* infection: the importance of replacement gilts in the infection dynamics and the genetic variability of *M. hyopneumoniae*.

It is known that piglets are born free of *M. hyopneumoniae*. That is, there is no transplacental transmission from the sow to the piglets. However, studies show that after one week of life, it is already possible to detect piglets positive for *M. hyopneumoniae* by PCR and that the proportion of positives over the lactation period increases due to direct contact between positive sows and their litter. Comparing different categories of sows, young females, especially gilts, are more likely to be detected positive for *M. hyopneumoniae* by PCR. In a study carried out by the Swine sector of UFRGS, it was observed that up to 15.7% of gilts possibly reach their first farrowing shedding the bacteria, representing risk in the transmission of *M. hyopneumoniae* to their litter. Another study also carried out by the same group found negative subpopulations of gilts in farms positive for *M. hyopneumoniae*. This result demonstrates that if not exposed to *M. hyopneumoniae*, the gilts can reach their first farrowing without having had the contact with this bacterium, turning them susceptible to future infections, increasing the risk of transmission of *M. hyopneumoniae* to their piglets not only in the first but also in subsequent farrowing. The only way to reduce these risks would be to acclimate gilts for *M. hyopneumoniae* immediately after their arrival on the farms.

An important aspect to be considered when practicing gilt acclimation for *M. hyopneumoniae* is the genetic variability of this organism. In our studies, we detected a wide range of *M. hyopneumoniae* variants in Brazilian multipliers, demonstrating that not all farms have the same profile. These results, associated with the limited cross-protection between variants of different degrees of pathogenicity, highlight the importance of carrying out acclimation management only in the farms that receive these animals and alert us to the risks of introducing gilts from external sources that are positive for *M. hyopneumoniae*.

The gilts acclimation for *M. hyopneumoniae* is the only management that guarantees the exposure of these animals to the bacterium. When exposed purposely at a young age, gilts have a greater chance of recovering the infection by the time of first farrowing, also reducing the formation of negative gilts subpopulations that may cause health imbalance in the herd in the future. Thus, the presence of acclimated gilts reduces the chances of transmitting the agent from sows to their litter, and consequently, the future transmission of *M. hyopneumoniae* between infected and susceptible piglets in the nursery and finishing phases, when clinical signs and injuries are commonly observed.

Etiology of Pneumonia in Commercially Slaughtered Pigs

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In technified pig production, the losses caused by pneumonia in the finishing phase are significant, and few studies perform the etiological characterization of the agents involved. Thus, the objective of this work was to evaluate the causes of pneumonia in pigs slaughtered commercially. Thirty lungs, with lesions suggestive of pneumonia, were collected from five slaughterhouses belonging to different agro-industries. Of the 150 lungs evaluated, the mean lung injury score was 2.2, varying from 1.53 to 2.83 among slaughterhouses. The most frequently histopathological findings were lesions suggestive of coinfection by Influenza A virus (IAV) and *Mycoplasma hyopneumoniae* (Mhyo), corresponding to 54.7% (82/150), whereas *Pasteurella multocida* type A (PmA) was isolated from 54.9% (45 / 82) of these cases. Other frequent results were the presence of histopathological lesions suggestive of infection only by Mhyo (25.3%; 38/150) and lesions suggestive of infections involving only IAV (9.3%; 14/150). In 103 samples (68.7%) it was pointed out that more than one infectious agent involved. These findings could explain the severity of macroscopic lesions since mixed infections tend to cause more severe pneumonia. Although IAV infection is often associated with younger animals, in the present study, 64.7% (97/150) of the lungs of slaughter pigs presented histopathological lesions suggestive of IAV, ranging from 20% (6/30) to 86.7% (26/30) among the evaluated slaughterhouses. Regarding the chronicity of histopathological lesions suggestive of IAV, of the 97 samples, 17.5% (17/97) presented acute, 29.9% (29/97) subacute, and 52.6% (51/97) chronic lesions. The 46 samples suggestive of subacute and acute IAV infection were selected for evaluation by IHC and RT-qPCR for IAV. A total of 16 samples (34.9%; 16/46) presented positive IHC scores. In the RT-qPCR, six samples (13%; 6/46) were positive for IAV. The divergence between the number of positives in the IHC and the RT-qPCR may be related to RNA denaturation, which can easily occur during sample processing, and also to the short time for viral detection in the animal organism. In the bacteriological examination, 43.3% (65/150) of the samples presented pure PmA isolation, which ranged from 0%

(0/30) to 66.7% (20/30) among the slaughterhouses. In the present study, PmA was isolated in cases of pneumonia with histopathological lesions suggestive of infections by IAV and/or *Mhyo*, which corresponded to 42.7% (64/150) of the lungs evaluated. A total of 79.3% (119/150) of the samples were positive for *Mhyo* in the RT-qPCR. From those, 84.9% (101/119) had lesions suggestive of *Mhyo* in histopathology. The results of this work indicated the high frequency of mixed infections, mainly caused by *Mhyo*, IAV, and PmA, and the elevated detection of IAV lesions in the lungs with pneumonia lesions in finishing pigs. Considering that three weeks after IAV infection, the injured tissue recovers, and it is not possible to observe lesions by histopathological examination, it is suggested that histological lesions classified as acute, as well as subacute and chronic lesions, occurred at the end of the finishing phase.

05

Innovation and Research:

abstracts presented in the panel "Enteric Health"

Characterization of salmonellosis outbreaks in swine in Brazil

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Production diseases are responsible for major economic losses in intensive pig farming. Among them, salmonellosis stands out from the clinical perspective and also from a food safety point of view. Among 2,600 known Salmonella serovars, the cases of clinical salmonellosis in pigs are usually associated with two main serovars: *Salmonella* serovar Choleraesuis that causes severe, invasive, and septicemic disease, and *Salmonella* serovar Typhimurium associated with cases of enteritis. In Brazil, since 2013, the number of clinical cases of salmonellosis in the principal producing states has been gradually increasing. To know the epidemiology of the disease, 130 outbreaks of salmonellosis, which occurred between 2011 and 2017 in ten Brazilian states, were characterized by field information and investigation of a Salmonella isolate from each outbreak.

Results of serotyping showed that 42.31% (55/130) of the isolates belonged to the monophasic variant of the serovar Typhimurium (4, [5], 12: i :-), followed by 35.40% (46/130) of the serovar Choleraesuis and 10.77% (14/130) of the isolates were *S.* Typhimurium. Other serovars such as *S.* Rissen (3/130), *S.* London (2/130), and *S.* Panama (2/130) were identified in smaller numbers. In addition, a single strain belonging to the serovar was identified*: S. anatum*, *S. bovinsmorbificans*, *S. derby*, *S.* Group D, S. Group E4 (O: 19 :-), *S. infantis*, *S. newport*, and *S. oslo*. The occurrence of the monophasic variant of *S.* Typhimurium, previously classified by serotyping, was confirmed by PCR Multiplex.

Outbreaks were classified into four clinical-pathological categories according to the Salmonella isolation site. Of the 130 cases, 128 contained information regarding the isolation site, and of these, 50 were classified as enteric, 48 septicemic, 17 invasive hepatobiliary, and 13 nodal or enteric nodal. Regarding the clinical presentation of the serovars found, it was observed that 88% (44/50) of enteric cases were associated with the serovar Typhimurium and its monophasic variant. Regarding septicemia, 75% (36/48) of the cases were associated with the serovar Choleraesuis. The production phase in which the disease occurred was reported in 114 cases. Of these, eight occurred during pre-weaning, 53 cases occurred in the nursery, and 53 in the

growing and finishing phase.

Regarding antimicrobial resistance, 113 isolates (86.92%) were classified as multidrug-resistant, presenting resistance to three or more classes of antimicrobials. A high frequency of resistant isolates was observed for tetracycline (90%), followed by florfenicol (77.69%), doxycycline (76.92%), gentamicin (73.84%), colistin (63.07%), and streptomycin (62.30%). In contrast, 88.46% of the isolates were sensitive to fosfomycin followed by lincomycin/spectinomycin (93.84), ceftiofur (86.92%), and norfloxacin (86.92%).

The genotypic relationship between the isolates was investigated using the pulsed-field gel electrophoresis technique. A large clonal group of *S.* serovar Choleraesuis with 41 isolates was widely distributed in six pig-producing states: Goiás, Minas Gerais, Paraná, Rio Grande do Sul, Santa Catarina and São Paulo. Also, 14 isolates belonging to the same group had the same antimicrobial resistance profile (Dox., Ffc., Gen., Str., Tet.), and a further eight isolates contained this same conserved profile plus other antibiotics. On the other hand, the monophasic variant of serovar *S*. Typhimurium presented eight groups with great diversity in the phenotypic profile of resistance to antimicrobials.

This study revealed that outbreaks of salmonellosis are occurring endemically in the states with the highest pig production in Brazil. The nursery, growing, and finishing phases are mainly affected, but it can also occur in the farrowing. The most prevalent serovar was the monophasic variant of *S*. Typhimurium (4, [5], 12: i :-), which emerges as an important serotype that causes clinical disease in pigs. In addition, Typhimurium and Choleraesuis serovars were identified and showed a high rate of multidrug resistance to the main antimicrobials used in the field. Regarding genotypic evaluation, a majority clonal group of *S*. Choleraesuis associated with a specific antimicrobial resistance profile suggests that similar strains are circulating in different regions of Brazil.

Prevention of neonatal diarrhea due to *Clostridium difficile* by competitive exclusion

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Clostridium difficile infection (CDI) is today one of the principal causes of diarrhea in newborn piglets worldwide, including Brazil. In addition, studies have demonstrated the potential transmission of *C. difficile* between animals and humans. Despite the recognized impact on pig production and suspected to be a zoonotic disease, there are no specific products, including vaccines, for the prevention and control of CDI in the world market. So far, control is done exclusively by management measures that are known to be of low efficiency. One of the most promising prevention approaches to date, and widely tested in humans, is called "competitive exclusion": it consists of providing non-toxigenic strains of *C. difficile* orally. These colonize the intestinal tract and prevent, by competition, the colonization by strains of *C. difficile* capable of causing disease. Thus, the objective of the present study was to evaluate the protective potential of a non-toxigenic strain of *C. difficile*, named Z31, against CDI in swine. The project, started in 2008, was divided into three phases. In phase 1, the genotypic and phenotypic characterization of strain Z31 was carried out, including the viability of the product at different storage temperatures. Genomic sequencing of Z31 revealed the presence of genes responsible for spore production and stability, intestinal adherence, and biofilm formation, but confirmed the inability of the strain to produce the disease thanks to the absence of genes encoding toxins A and B. For large-scale production, different usual culture media were evaluated, allowing concentrations of almost 10^7 CFU/mL with a proportion of spores greater than 98%. In addition, strain Z31 maintained the viability of up to 2 years when stored at room temperature, indicating great ease of production, storage, and distribution of the product. In a second phase, the preventive capacity of strain Z31 against CDI was evaluated in a hamsters experimental model, widely used for the evaluation of vaccines and probiotics that aim to prevent diarrhea by *C. difficile*. Strain Z31 was able to protect 100% of the

animals in this test, accrediting it for tests on newborn piglets. In the final stage, the preventive capacity of the Z31 strain in pigs in a controlled model and then in a naturally infected commercial farm. Z31 prevented CDI in the swine model, reducing clinical signs, macro and microscopic lesions and fecal shedding of the pathogen in the swine-controlled model. In the naturally infected commercial farm, even in the presence of other enteropathogens, Z31 substantially reduced the occurrence of CDI, the fecal shedding of toxigenic *C. difficile*, and the occurrence of neonatal piglets diarrhea. In conclusion, strain Z31 was able to prevent CDI in pigs and demonstrated desirable characteristics for its potential commercial use.

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Lawsonia intracellularis: mechanisms of cellular invasion and permissibility of macrophages

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Lawsonia intracellularis is a obligate intracellular bacterium, microaerophilic, Gram-negative, that causes proliferative enteropathy (EP) (1). Endemic disease in the global pig herd (2).

The pathogenesis of *L. intracellularis* is still poorly studied and understood. The infection occurs via the fecal-oral route. In the gastrointestinal tract, the bacterium survives the hostile environment of the stomach by enzymatic mechanisms (2) and infects enterocytes in the intestine, most of the time, initially in the ileum, however, all portions of the intestine are susceptible. Upon contact with enterocytes, *L. intracellularis* is internalized. The mechanisms of bacterial endocytosis are two principal: a) zipper mechanism - dependent on the activity of the eukaryotic cell, which will recognize the bacterium by receptors present on the cell's cytoplasmic membrane and then internalize it by signaling a protein, called clathrin (3); b) trigger mechanism - dependent on the activity of the bacteria that will secrete in the cytoplasm of the host cell, effector proteins that will induce the eukaryotic cell to perform the endocytosis process (4). The endocytosis mechanisms of *L. intracellularis* still need to be confirmed.

Another issue related to the pathogenesis of *L. intracellularis* that still needs further studies is how much the bacterium survives inside macrophages. Controversial data exist in the literature (5, 6) despite no studies with the central objective of evaluating the interaction of macrophages and *L. intracellularis*.

Thus, this description details two studies, both *in vitro*, which aimed to evaluate the mechanisms of *L. intracellularis* endocytosis and the interaction of *L. intracellularis* with swine macrophages.

The cells used were IPEC-J2 (isolated porcine enterocytes cells) and macrophages obtained from peripheral blood (PB). Mononuclear cells (monocytes and lymphocytes) and monocytes differentiated into macrophages were obtained from peripheral blood and used for the study.

The *L. intracellularis* strain used in the studies was the reference strain (PHE-MN01).

Genetic modulation techniques (interference RNA) were performed to decrease the expression of clathrin to assess clathrin-dependent endocytosis of *L. intracellularis*. Western blot was performed to prove the effectiveness of genetic modulation. Confocal microscopy was performed to assess the co-localization of *L. intracellularis* and clathrin. For quantification of the endocytosed *L. intracellularis*, real-time PCR was used.

Transmission electron microscopy was performed to assess the interaction between *L. intracellularis* and macrophages in an observational proof of concept study.

L. intracellularis was observed co-located with clathrin under a confocal microscope; however, the decrease in clathrin expression (confirmed by *Western blot*) did not statistically reduce the internalization of *L. intracellularis*. Thus, a new trial was carried out. Dead *L. intracellularis* was placed in contact with IPEC-J2, and it was demonstrated in this second essay that the decrease in clathrin expression was determinant to decrease the internalization of *L. intracellularis* significantly.

Transmission electron microscopy demonstrated the presence of viable *L. intracellularis* within phagolysosomes and also bacteria in a free binary division in the cytoplasm.

Conclusions

• *L. intracellularis* is endocytosed by clathrin-dependent mechanisms and/or by active mechanisms of the bacterium itself.

• *L. intracellularis* can survive in the phagolysosomal environment. Also, L. intracellularis has been shown to proliferate freely in the cytoplasm of macrophages.

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Effect of the co-infection of Lawsonia intracellularis and *Brachyspira hyodysenteriae*

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Among the major diseases that cause diarrhea in the final stages of pig development, we can highlight swine dysentery and proliferative enteritis. Swine dysentery (SD) is characterized by severe fibrous mucohemorrhagic colitis, and its primary agent *is Brachyspira hyodysenteriae*. Lesions are restricted to the large intestine, resulting in dehydration and death in untreated animals.

Porcine proliferative enteropathy (PPE) has *Lawsonia intracellularis* as the causative agent. It has a clinical presentation in acute, chronic, and subclinical form. The chronic form affects young animals between 6 and 20 weeks, presenting anorexia and diarrhea. The acute form is characterized by hemorrhagic enteritis with sudden death seen in older animals and replacement gilts. In the subclinical form, the identification of clinical pictures of diarrhea with intermittent elimination of *L. intracellularis,* associated with delayed growth is not clear.

The pathogenesis of the two diseases is complex and poorly elucidated. It is known that the diet and the microbiota have a strong influence on the occurrence of clinical signs. Some studies demonstrate the effects of different diets on colonization and the presence of microorganisms that are relevant to the establishment of clinical signs. Thus, when manipulating the environmental conditions, it is possible to control certain organisms.

In studies inoculating gnotobiotic animals with *B. hyodysenteriae* or *L. intracellularis*, the pigs do not develop a typical clinical picture of swine dysentery and proliferative enteropathy, respectively, showing less susceptibility to infection by these two agents depending on the present microbiota.

Regarding *L. intracellularis* and *B. hyodysenteriae*, there are few reports about the mixed infection by these two agents; however, there has been an increase in co-infection in the diagnostic routine.

This study aimed to evaluate the clinical picture, anatomopathological changes, shedding in the feces, and the intestinal microbiome compared to

individual infection.

It was selected 45 piglets aging five weeks, randomly separated into four groups: *B. hyodysenteriae* and *L. intracellularis* co-infection (CO), *B. hyodysenteriae* (BRA), *L. intracellularis* (LAW), and negative control (NEG) evaluated over 21 days. At 0 dpi, animals from the CO and LAW groups were inoculated with $2.76x10^6$ *L. intracellularis*/ml. Seven days after inoculation, piglets from groups CO and BRA received $5.31x10^6$ *B. hyodysenteriae* / ml for three days. The diarrhea score was assessed daily, and RT-qPCR was performed for *B. hyodysenteriae* and *L. intracellularis*. At 21dpi, the animals were necropsied and macroscopic lesions were evaluated, bacterial isolation for *Brachyspira* sp., immunohistochemistry and histopathology for *L. intracellularis* were done. Sequencing of the hypervariable V4 region of the 16*S* rRNA gene was performed using the "Fusion" method by Ion Torrent 16*S* Metagenomics kit, in day -5 and 21 dpi. The QIIME was used to generate results of α and β diversity.

As a result, clinical signs and diarrhea started at 12 dpi, affecting 11/12 animals and at 14 dpi, 5/11 animals in the CO, and BRA groups, respectively. Intermittent diarrhea was observed in only four pigs in the LAW group. The injuries were severest, with a significant difference for the CO group in all parameters evaluated in the large intestine. Inflammation, necrosis, hemorrhage, goblet cell hyperplasia, and total lesions were significant when comparing the CO to the LAW group. The BRA group differed only from the CO when comparing crypt abscesses and enterocyte hyperplasia occurrence. Mild to moderate staining was observed in CO and LAW in immunohistochemistry. *B. hyodysenteriae* was isolated from 11/12 animals from the CO group, and from 5/11 animals from the BRA group. Animals from the CO and LAW groups began to shed *L. intracellularis* in the feces after 3 dpi, and until the end of the study, all animals tested positive. 10 out of 12 animals from the CO group and 7 out of 11 in the BRA group, were tested positive for *B. hyodysenteriae* by RT-PCR, starting three days after inoculation with *B. hyodysenteriae*.

Regarding the fecal microbiome, it was possible to observe a greater relative abundance with a statistical difference in comparison with the other groups for the genus *Prevotella, Anaerovibrio, Bacteroides, Butyricimonas, Desulfovibrio, Fusobacterium*, and p75-a5 in the CO group, a greater abundance of *Clostridium* in the BRA group. In the LAW group, *Megasphaera* and *Dialister* were statistically the most prevalent and Odoribacter for the NEG group.

More work is essential for better understand the microbiota profiles associated with susceptibility to the disease. This is the first report that characterizes the picture of experimental co-infection. Clinical, macroscopic, and microscopic signs were significantly more severe in the CO group. In the LAW group, we observed the subclinical picture. Possibly, the infection by *L. intracellularis* caused initial damage that aid in colonization by *B.*

hyodysenteriae. It is relevant to mention the immunosuppressive mechanism already demonstrated in pigs with proliferative enteropathy, with limited infiltration of inflammatory cells during the development of lesions with negative regulation of the immune response.

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