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2018 International PRRS Symposium

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Healthy Pig
Safe Pork

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Local Organizers:
Chinese Association of Animal Science and Veterinary Medicine (CAAV)
China Agricultural University (CAU)

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PROCEEDINGS VOLUME I
KEYNOTE LECTURES & ORAL PRESENTATIONS

IPRRSS 2018 IPVS 2018
I

Acknowledgments

We sincerely thank all members of IPVS2018 Scientific Committee to review and evaluate all abstracts submitted to 25th International Pig Veterinary Society (IPVS) Congress. By their extremely efforts, we accomplished this comprehensive proceedings. Particularly we are grateful to Prof. Jun Han, Dr. Lei Zhou and Dr. Xinna Ge at China Agricultural University, who were responsible for communication with the reviewers, distributing and collecting abstracts, and making final check for all selected abstracts. We also express our thanks to all authors' contribution to the proceedings.

Preface

The International Pig Veterinary Society (IPVS) Congress is major objective of the IPVS, and already had 24 editions with success in past 50 years. In 2018, this great event will be held in Chongqing, China at the great moment of the 50th anniversary.

The Local Organizing Committee of China will follow up the tradition and format of IPVS Congress, but also paint colors of Chinese. The core value of Chinese philosophy is to create win-win situation. We would like to make this event in harmonious, efficient and easy atmosphere. We believe this international and professional Congress will create a platform for global veterinarians and pig producers to exchange experiences, introduce new development researches and solutions against current, and emerging and re-emerging diseases, and most important, to make friend and make memory in China.

There were totally 918 abstract submissions from 40 countries, 165 abstracts were selected for oral presentation and 738 for poster presentation. Those selected abstracts, together with invited speaker's abstracts, will be collected in IPVS2018 proceedings.

I am grateful to all authors and invited speakers to contribute to this proceedings and scientific programme during IPVS Congress. I wish all guests can take away the valuable things from this very informative and fruitful Congress.

Prof. Hanchun Yang

Chairman of IPVS 2018 Scientific Committee

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Locations of IPVS Congresses

1969 Cambridge, UK
1972 Hannover, Germany
1974 Lyon, France
1976 Ames, USA
1978 Zagreb, Yugoslavia
1980 Copenhagen, Denmark
1982 Mexico City, Mexico
1984 Ghent, Belgium
1986 Barcelona, Spain
1988 Rio de Janeiro, Brazil
1990 Lausanne, Switzerland
1992 The Hague, Netherlands
1994 Bangkok, Thailand
1996 Bologna, Italy
1998 Birmingham, UK
2000 Melbourne, Australia
2002 Ames, USA
2004 Hamburg, Germany
2006 Copenhagen, Denmark
2008 Durban, South Africa
2010 Vancouver, Canada
2012 Jeju, Korea
2014 Cancun, Mexico
2016 Dublin, Ireland
2018 Chongqing, China

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Keynote Speakers

Emerging porcine diseases – the drivers and the dogma

Trevor W Drew, OBE
Animal & Plant Health Agency, Weybridge, United Kingdom
In memory of Dr. T. J. L. Alexander

I am greatly honoured to present the Tom Alexander Memorial Lecture today. Professor Alexander's contribution to pig health has been both cutting edge and highly practical. Being a fellow Englishman, our paths crossed on one or two occasions through our mutual pursuit of our shared interest in pig health, ranging from the generic subject of biosecurity and risk pathways, the emergence of PRRS virus, to introduction to the early advances in xenotransplantation and inherent problems posed by endogenous retroviruses.

I would like to thank my colleague and "GeMen", Professor Yang Hanchun, for his kind invitation to present this lecture today. My objective is to explain something of the scientific mechanisms that drive pathogen evolution, the biological and environmental drivers that impact on this process and the alternative futures we face, based on the road we take.

In my memorial lecture today, I would like to focus on emerging and re-emerging viral diseases, asking what environmental and pathogen traits might drive this emergence, with an exploration of the mechanisms behind viral evolution and concluding with a more speculative look at what the future may hold and what, if anything we can, and should do about it.

There has been a significant global increase in pig production over the last 30 years, also of greater intensification of pig-rearing and increasing international movement of products, in terms of their source, destination and diversity. In parallel, here are also seemingly ever-greater numbers of novel and re-emerging pig viral diseases to challenge the industry. Is this coincidental, or are they linked – and if so, how?

In some cases, such as for African swine fever, outbreak reports and our awareness are certainly related to its incursion to new areas, rather than truly "emerging" as a novel pathogen. But, taking this specific example, why have we not been able to control it? Certainly, historical introductions into Europe and South America have been controlled, so why does it seem so present such a challenge today? So let us examine some oft-cited drivers for viral emergence and increasing pathogenicity:

Intensification of production

Pig production figures show an increasing trend in global pig production, with China producing more than 53 thousand metric tons of pork in 2017 [4] – more twice as much as the European Union and nearly five times as much as the United States.

An obvious element of intensive pig production is the mere density of animals, also coupled with homogeneous genetics, age, and that large-scale production invariably involves dedicated facilities on multiple sites, sometimes also with separate industry producing custom feed – although sometimes also commercially integrated into the pig production system itself. The industry today is increasingly dominated by large international corporations, operating as individual or multi-site integrated units, sometimes with separate ownership, operating units and geographical locations for each phase of production. For some pathogens, such intensification may be a potent driver for increased pathogenesis and pathogen load – and I shall provide an example of this later in my talk.

In these integrated systems, movements of pigs and germplasm play an important part. An analysis of international exchanges of animal genetic resources from 1990 to 2005 [5] noted that 95% of the flow was from Europe and the United States to developing countries, with a dramatic increase seen between 2000 and 2005. The effect of this flow of genetic resource is the displacement the indigenous animal genetic resources of developing countries along with a resultant increasing level of genetic homogeneity among global pig populations.

In the early 2000's, there were also some concerns about the lack of diversity of the genetics of imported pig breeds to

China, over time. These now seem to have abated – likely through more controlled breeding and imports of germplasm. Tang and others [6] noted some improvement in diversity of Chinese Landrace and Yorkshire, but increasing loss of diversity among Duroc lines.

The impact of genetic bottlenecks on disease emergence or prevalence is hard to judge, but the concept of "hybrid vigour" is well known in the context of disease resistance and this trend to a porcine "monoculture" may be to the detriment of natural resistance to pathogens. And it may even provide opportunities for emergence and more rapid spread of newly emerging pathogens.

A correlate of intensification of pig production is a significantly increased demand for animal feed. Based on projected increase in livestock numbers (including pigs) and the move from backyard and small farms to larger production units, animal feed production will need to grow significantly to meet future demand. Already, nearly half of global agricultural land is used for producing livestock feed and more than 20% of wild-caught fish is fed to animals. Feed production currently contributes to 45% of greenhouse gas emissions from livestock production, and much of it comprises high-value foods rich in protein and nutrition, such as soy and maize. It is no coincidence that production of these two crops has shown the greatest increase over the last 20 years and is predicted to continue. Figures for Russia alone suggest a forecast a rise in pig feed production of 32% between 2016 and 2021, while poultry and cattle feed forecasts are lower, at 18% and 4% respectively. It is unclear whether such an increase is economically or environmentally sustainable, especially given the increasing competition for grain, for use in biofuel production. The projected impacts of climate change are worthy of a separate talk, but it is predicted that heat and water scarcity will have a direct impact on animal health and are also predicted to reduce the quality and supply of feed and fodder [7].

For its part, the societal impact on smallholder farmers is the subject of much concern, and often given little consideration. In poorer countries, pigs play a very valuable role in subsidising income – acting as a "piggy bank" for dowries, school fees, medical expenses etc.

Expansion of production to new areas

The link between disease introduction and spread, and contact between domestic pigs and wild suids is nothing new, and has been a key driver in the move towards larger production units with increased biosecurity. However, at a high level, there is increasing interest in pig production in many parts of the world, as a means of societal eco-development. In Africa, it is being seen as a key tool in poverty alleviation for poorer sectors, but disease and poorly established processing and markets are holding back development. Nevertheless, pig numbers in Africa are increasing sharply, most significantly in Nigeria, Malawi, Angola and Uganda [8]. This production is largely within backyard and small-scale operations, with little in the way of farm biosecurity, so it has been inevitable that cases of African swine fever and other diseases are common, through contact with bushpigs, forest hogs and warthogs, as well as feral pigs. Whilst it is unlikely that such countries will officially export pigmeat and products in the near future, it does pose an increased risk to free countries through passenger carriage and possibly via airline food.

In SE Asia, production has been maintained or with slow rise in current areas, also with development in new regions – with steep rises in Viet Nam and Myanmar [8]. An increasing trend towards siting farms in remote areas as a biosecurity measure, also has inherent risks, as exemplified by the emergence of Nipah virus in Malaysia in 1999, with agricultural expansion and intensification identified as underlying causes of its emergence [9, 10].

However, despite the significant rises in pig numbers seen in some countries over the last five or 10 years, at a global level, this rate of expansion in production is predicted to slow over the next decade [11]. In China, production has declined slightly over the past four years, mainly attributed to the implementation of new environmental protection law, which restricts pig production in the south of China, also with incentives to transfer production in the north. Strong production growth rates over the outlook period are also predicted in Brazil, Mexico, the Philippines, the Russian Federation, the United States, and Viet Nam. The strong import demand from China is also anticipated to stimulate marginal growth in production in the European Union despite a saturated domestic market.

This increased production in some countries will inevitably increase the demand for feed and for more innovative ways of

feeding pigs – particularly in developing countries.

Integrated animal production

The popularity of small-scale pig production is primarily down to the ease with which it integrates into the other activities of such farmers, with feed largely based on crops and by-products, manure providing a source of fertiliser and, latterly, biogas for local use.

The famous “black pig” of Jeju island was renowned for its flavour, but few appreciated that its husbandry involved the feeding of human excrement and it is unsurprising that this practice ceased many years ago, when the risks to human health became apparent. Nevertheless, the practice still goes on, albeit opportunistically, among scavenging pig populations in many parts of the world.

What is less well appreciated is that other systems of “integrated animal production” have also been developed and promoted globally, also gaining formal support from international agencies.

In the 1990s, the idea of integrating fish production with that of pigs and poultry gained much popularity, with potential disadvantages rarely given voice – the production statistics were too compelling. And when risks were assessed, it was considered that the fish themselves posed little risk, except perhaps as receptacles for parasites.

Today, we appreciate much more the risks posed to wildfowl which may visit such ponds, with the attendant risks of co-infection and transmission of influenza viruses and this past practice has been cited as a possible contributor to their current pathogenicity.

An early analysis by FAO [12] drew the conclusion that avian influenza pandemics were unlikely to be generated from integrated fish/poultry farming, since pigs, at that time, were regarded as essential "mixing vessels" for the reassortment and/or recombination of poultry viruses. They claimed that, since pigs were rarely reared with both poultry and fish (except in certain parts of Asia), the risks were minimal, especially compared to the raising of pigs and poultry on shared backyard premises, which is common in the region. However, this analysis did not consider fish/pig farming - we now know that the practice of pig/fish rearing is widespread and also the apparent constraint of pig involvement is now void anyway, since current HPAI H5N1 can pass directly from poultry to humans.

The risk posed by this practice is currently unknown, but, given that wildfowl are known to be efficient spreaders of disease, the practice certainly represents a theoretical risk as a potent driver for facilitating influenza virus reassortment and dissemination.

It is not the purpose of this paper to examine the pros and cons of smallholder vs intensive pig production per se – there are numerous papers on this subject. What I would like to do is to consider the environment presented by these different systems and the potential effects they might have on how viruses evolve and adapt.

How to viruses adapt to environmental change?

Before launching into a discussion of some key pig viral diseases, let us first briefly review the concept of viral diversity and evolution. Obviously, the attributes of a virus are encoded within its genome. What is less obvious is that the genome of a virus is never fixed and any virus population comprises a mixed population of nucleotide sequences. For DNA viruses, this population is rather constrained, because of the cellular proof-reading mechanisms that exist for DNA replication, which also ensures high fidelity during DNA virus replication. But for RNA viruses, proof-reading generally does not occur, or, if it does, the efficiency is less than for DNA viruses - so there is a much higher mutation rate. As a consequence, RNA viruses exist as a “cloud” of variants, consisting of the total number of variations that can exist for any given viral genome. This “quasispecies” concept [13] is not just a mathematical theory – it has a real basis in the biology of viruses and, in particular, of RNA viruses [14, 15]. Although quasispecies theory has been around for more than 50 years, it’s relevance to viral evolution and field pathogenicity is still generally poorly understood by clinicians. In biology, the constraints and disadvantages of asexual reproduction are well recognised, so it is not surprising that mechanisms have evolved whereby viruses are able to adapt to new environments – indeed, it can be argued that those which did not are already extinct! These mechanisms of mutation and recombination we see in virus replication have even been described as the evolution of “virus sex” [16, 17].

Within a viraemic animal infected with an RNA virus, there exists a population of viruses or "mutant spectrum", generated by the replication process and whose genetic makeup is therefore subtly variable. Of course, there are a large number of constraints placed on this variation. Many variants with changes in critical areas will be non-viable, by virtue of changes that render key elements of the infection process defective. But likewise, there is a selection process at work, which can tolerate certain changes, provided other changes also occur. And, in some cases, there might be a change which is potentially advantageous to the replication and dissemination of a particular virus clade within the mutant spectra, either within the environment that the virus is currently in, or within a new environment. Studies of foot and mouth disease virus have demonstrated that quasispecies diversity can be detected in new environments presented by different host species [18], between different animals in the same herd and even within different tissues of the same animal [19].

And we should not completely discount the role played by "defective" virions in the cocktail of variants that comprised the mutant spectrum. Initial studies of these defective particles focused on their ability to interfere with replication, earning them the title of "defective interfering (DI) particles". And it is certainly true that a number of studies have demonstrated that prior exposure to such particles can afford some level of protection from infection [20]. For classical swine fever, the presence of DI particles has been associated with a cytopathic phenotype, which is considered to be a marker of attenuation [21, 22] but there is also evidence that, within the mutant spectrum generated by HIV infection, such particles can actually contribute to pathogenesis [23].

This generation of mutant spectra ensures that the evolutionary process is constant. For viruses that have been present in pigs for many years, the relationship tends to be more fixed, resulting in the emergence of one or more stable ensembles of the genome – more often referred to as genotypes or serotypes. Even so, each ensemble will itself consist of a variant mutant spectrum, with the viral sequences of particular isolates defining only the major population within the mutant spectrum present at that time, in that tissue of that animal.

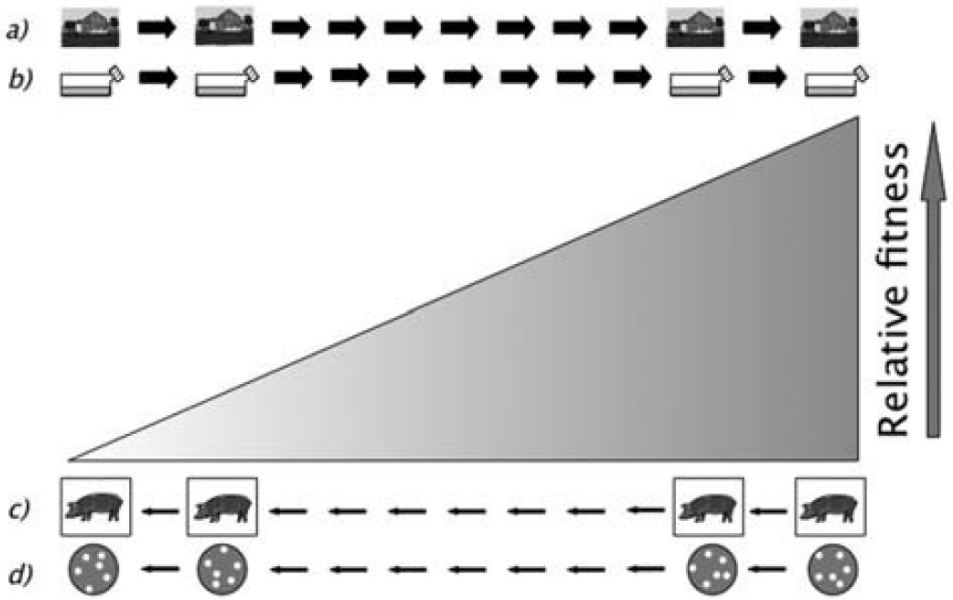
These RNA viral populations will constantly be subjected to changes in the way pigs are raised, so providing new environments that may select new populations of virus variants. Pig immunity can also be considered an environmental factor and can be affected at a number of levels, including genetics, age and maternal factors, nutrition, pollutants, vaccination regime, stress and the presence of other viral, bacterial and other pathogens. All these factors can affect the ability of a viral pathogen to cause infection and disease, and also ultimately influence the makeup of the progeny viral population that may emerge from the infected animal.

The relative ability of a particular virus population to replicate under defined environmental conditions is defined as its "fitness" [15]. This is a complex biological parameter, which varies, depending on the amount of virus that is present. This is because the two primary factors – random shift (the replication errors and recombination) and positive selection (the ability of all members of the diverse population to reach a new host cell) are both influenced by the size of the replicating population and the availability of susceptible hosts. Transmission of large populations often results in fitness gain, whilst repeated bottleneck events lead to average fitness losses [24-26]. This can be nicely demonstrated in the laboratory, using two simple but very different methods of producing virus stocks *in vitro* – essentially measuring difference in fitness gain by the speed and amount of progeny virus produced. Serial passage in cell culture – by repeatedly taking supernatants from one cell culture onto fresh culture – can results in profound fitness gain [27], whilst repeat plaque purification – taking a restricted virus population from a single virus plaque – results in and accumulation and fixation of genetic mutations, resulting the bottleneck effects of Muller's Ratchet and fitness loss [25, 28, 29] see Figure 1.

This fitness loss has also been described *in vivo* for a number of viruses, for example during serial passage of low levels of foot and mouth disease virus in animals [30]. However, it should be stressed that fitness gain is only in the context of the specified environment – high fitness gain *in vitro* may not translate to highly productive infection *in vivo.*

Once can therefore postulate that the environment provided by high density pig production would strongly favour *in vivo* fitness gain at farm or production chain level, in the same way as is seen with large volume *in vitro* cell monoculture passage in the laboratory - and may lead to increased replicative and infectious efficiency on the part of viruses replicating within such a system, possibly also with higher pathogenicity, since similar susceptible hosts are plentiful and in high density, so rapid death is unlikely to compromise transmission. In contrast, village pig production may result in

fitness loss, in the same way as *in vitro* plaque purification, manifesting as lower virulence viruses, with slower infection profiles and lower viraemia, due to a series of bottleneck events.



Adapted from [2]**Figure 1. A simplified schematic of fitness variation of a virus following replication in different environments**
In vivo passage in two different farming systems imposes the same opportunities for fitness gain and loss as their *in vitro* counterparts.
Large population passages (a = large pig farms with pigs of homogeneous genetics and age and b = sequential passage in monolayer cell culture) result in fitness gain. In these circumstances, random shift is minimal and mutant viruses with higher replication efficiency will predominate.
Passages involving very small amounts of virus, and/or with sequential bottleneck events (c = transmission between small isolated village pig populations and d = plaque picking) tend to result in fitness loss. Under these conditions, random drift will tend to prevail and genomes with higher replication efficiency will have little chance of becoming dominant in the quasispecies population. The population size needed to initiate a changed level of fitness within a given system will depend on the initial fitness of the virus population for the environment. [31].

The influence of "mutant spectra" in virus evolution

The evolutionary advantages of a virus population existing as a spectrum of subtly different genomes is intuitively obvious – that there is, encoded within the variants, the opportunity to benefit from a mutation, either with the existing environment or within a changed one. Domingo et al [31] have defined five parameters as key to adaptability: average number of mutations per genome; virus population size; genome length and mutations needed for a phenotypic change. Mutations that may be phenotypically relevant (i.e. change some viral characteristic) have the potential to strongly influence the development of new mutant distributions in an environment that favours them. The mechanisms that generate these mutations are not simply the by-product of error-prone replication, but have been positively selected and preserved, by virtue of conferred evolutionary advantage, either to the existing environment, or to any new one.

For viruses with the ability to generate only restricted diversity within their mutant spectrum, those with high relative

fitness will tend to preserve a dominant clade, but with minor variants also within the spectrum. Such viruses tend to have a relatively stable relationship with the host, but may be vulnerable to significant environmental change. Viruses with restricted diversity and also with low relative fitness may continue to infect their host, but are at risk of extinction. Viruses which have the ability to generate widely diverse mutant spectrum and have a constituent clade with high relative fitness, will also appear relatively stable in the short term, but may be able to adapt in response to environmental pressures such as vaccinal immunity and may generate different dominant clades in different tissues of the host. These viruses with a widely diverse mutant spectrum and also with low relative fitness may show the greatest ability to adapt to new tissues and/or even new hosts, irrespective of environmental changes, because the comparative relative fitness among variants within the spectrum is small, so and small advantage may result in a new clade becoming dominant. Such viruses may have a very restricted host range – or range of cells within a host, but show an extraordinary ability to evolve in response to immune and vaccinal pressures – and possibly may also readily adapt to new hosts. See Figure 2.

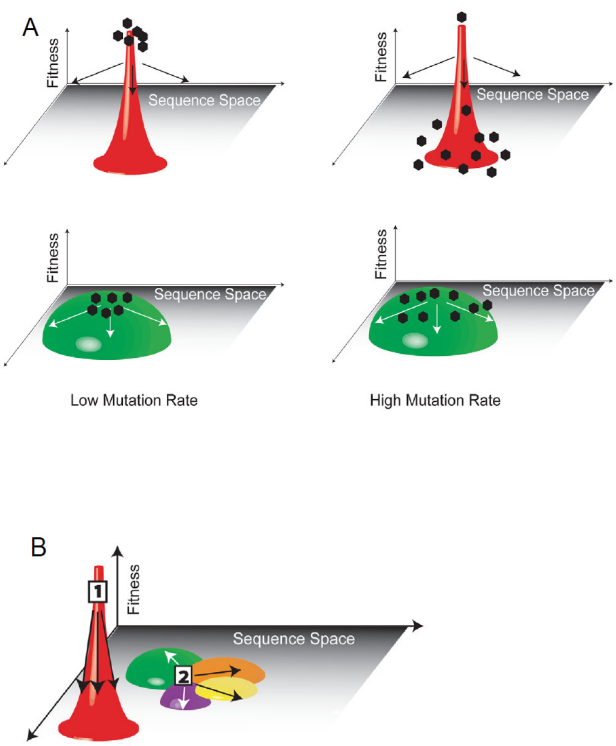


Figure 2. The fitness landscape and "survival of the flattest".
(A) Virus populations can be of high or low relative fitness for the environment in which they exist and also exhibit high or low mutation rates. At low mutation rates (right), no matter what their fitness, variants will be genotypically stable and cluster at the top of the fitness peak. The variant with the highest fitness will easily outcompete all others. At high mutation rates (left), variants spread out over the corresponding peaks. Variants on the flatter peak (bottom right) remain near their fitness optimum and have a higher mean fitness than the population located on the steeper peak (top right). The flatter population will prevail.
(B) Population 1 has high fitness but is trapped in sequence space because any mutation leads to a dramatic loss of fitness. Population 2 is more mutationally robust because mutation leads only to minor relative fitness loss. This flatter population is ideally situated to move through sequence space and access other local peaks through neighbouring mutational networks. Such viruses are better able to adapt to environmental changes, or new environments. This gives them a greater ability to persist in the host, evade vaccinal immunity and evolve to infect new cell types – or even new hosts.

Mutant spectra can also exert a modulating effect in some circumstances. Even if a particular mutant virus has the capacity to become dominant within a quasispecies, it may not necessarily do so. Its dominance will depend on the mutant spectra that surround the mutant. If an inferior mutant is surrounded by a large number of closely related mutants, in contrast to a fitter mutant that is either unique or with low numbers of related mutants, the inferior mutant will dominate [32]. This is a difficult concept to intuitively understand, but can be proven mathematically, and has also been observed *in vivo*. Probably the best cited example is that of polio vaccine, where it was discovered that only when a minority virulent poliovirus was above a certain defined concentration in a preparation, could it overcome the attenuated poliovirus vaccine strain (of inferior relative fitness) and cause neurological disease in vaccine recipients [33]. Vignuzzi and others [1] also demonstrated the cooperative nature of the quasispecies, in an elegant in vivo experiment which demonstrated that a

neurovirulent clade of poliovirus only infected the brains of mice when introduced as part of a diverse quasispecies population - see Figure 3.

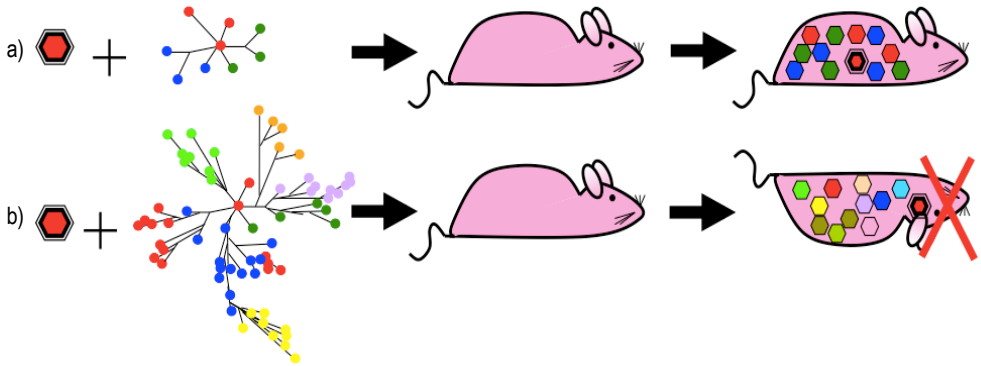


Figure 3. Quasispecies interactions can contribute to pathogenesis [1]. A lethal, neurotropic strain of poliovirus with a polymerase gene with varying fidelity that could be altered *in vitro* was cultured to produce a) a very restricted and b) a very diverse mutant spectrum. When intravenously injected into mice at the same multiplicity of infection, the restricted clade a) was infectious, but did not kill the mice and no poliovirus was isolated from the brains. In contrast, mice infected with the diverse clade b) died and the neurotropic strain was isolated from the brains of the mice. This observation of cooperative interactions between different individuals within the quasispecies provides a rationale for the role of quasispecies diversity in infectivity and pathogenesis.

The findings of this work are potentially profound and may explain why field infections with viruses are sometimes difficult to reproduce. Taking the example above, certain variants within the population may facilitate the colonization of the gut, another set of mutants may serve as immunological decoys that trick the immune system and yet another subpopulation may facilitate crossing the blood-brain barrier. One could even regard this concept as a modification of Koch's postulates – teaching an old dogma new tricks!

This interplay among the constituents of the mutant spectra within the quasispecies population results in a constant flux, with changes in the environment – such as introduction to new populations of host – providing an additional variable that may lead to the emergence of new dominant spectra. This is the backdrop behind the theatre of disease emergence or re-emergence and why viral diseases seem to gain or lose importance over time.

How does this translate into the diseases we face today?

The viral diseases that pose a continual challenge to global pig production have never been more diverse. Some diseases such as classical swine fever are successfully controlled in endemic regions through vaccination, but still pose a major challenge in some areas of the world and a constant threat to free countries. The diversity of the virus, though existing as three genotypes and several subtypes, does not seem to provide any particular advantage, with immunity being rapid, broad and T-cell mediated.

But there are also viruses newly emerged, that have surprised and confounded us. Some have been contained, others have become newly, or re-established as major pathogens of high economic importance. In many cases, they may have been known for decades, while for others, their emergence is still unknown. No matter their source, international trade seems to play a critical role in their distribution, which, in many cases, provides new environmental drivers towards increasing diversity. Let us examine two examples as illustrative of the concept:

Porcine reproductive and respiratory syndrome virus

There is probably little doubt that, for the pig industry, the single most important recently emerging disease is porcine

reproductive and respiratory syndrome (PRRS). The disease was first described in Canada in 1987, also spreading rapidly south into the United States. The first outbreak in Europe was in Munster Germany, in 1990 and later that year, Dr Gert Wensvoort and colleagues at Lelystad, Netherlands, were the first to describe the causative virus, PRRS virus (PRRSV) [34]. It soon became apparent that the causative viruses in the two continents were very different, though clearly of the same family [35, 36]. Originally termed "European" and "North American", these geographical labels are no longer appropriate, given the international spread of the disease and they are now termed type 1 and type 2 respectively.

Within a very short time, the disease spread to Asia, either through pork or, more likely via live pigs or semen, where it became rapidly established in pig populations throughout the region. A particular feature of PRRSV is its ability to rapidly mutate and evolve. Initially, viruses within each of the two populations were relatively similar, though the type 2 strains were more diverse. But within two or three years, similar levels of diversity were seen with type 1 viruses and, today, consequent with more extensive analysis of Eastern European strains, the diversity of type 1 strains is considered equal to or greater than type 2 – indeed, there is even emerging evidence of a distinct clade in eastern Europe which may be regarded as a putative "type 3" PRRSV [37, 38].

But the evolution of type 2 viruses continues, with significant diversity evolving in many parts of the world. Of particular note was the emergence of a highly pathogenic variant of a type 2 virus in China, with subsequent spread throughout SE Asia. A molecular marker – a deletion in non-structural protein (nsp) 2 – characterises this variant [39], though it is unclear whether this deletion is directly related to virulence [40]. It is worthy of note that strain of PRRSV Type 2, with increased virulence have also been detected in th US, with similar, but not identical deletions in the same region of the genome [41, 42].

So why does PRRSV exhibit such a propensity for rapid evolution? There are certainly two main attributes that facilitate this – firstly, it is an RNA virus, with a consequent innate error-prone replicative process which itself generates quasispecies; secondly, as with all members of the Coronaviridae, the molecular mechanism of its replication involves a three-step process from a full length, positive strand RNA to a full length negative strand, from which are generated a number of subgenomic mRNAs. The possibility for further evolution is provided by the arrangement of the open reading frames (ORFs) within the genome of the virus. By utilising all three frames within the triplet code portions of the ORFs overlap each other, so that two amino acids, in two different proteins, may be encoded by a single nucleotide sequence. The triplet nucleotide code contains an element of redundancy, with many amino acids coded by more than one nucleotide triplet – often the third nucleotide, hence often termed a "wobble-base". However, for PRRSV, if a single nucleotide transcriptional error occurs in the overlapping region, there is a greater chance of at least one amino acid change and the error may induce two changes.

However, these attributes alone do not account for the dramatic evolution exhibited by this virus since its appearance in pigs. Other arteriviruses, such as equine arteritis virus and lactate hydrogenase-elevating virus of mice, do not exhibit such evolution or diversity. A likely explanation, at least in part, is that this is a novel virus to pigs and is still adapting. The density of pigs in Asia has possibly provided opportunity for numerous large population passages, so increasing the relative fitness of PRRSV for its host. The relative novelty of this virus for the pig host and the environment in Asia has led to the emergence of a more virulent form, also seeming to productively infect older pigs, which now seems to predominate and displace other strains of the virus in the region.

Porcine epidemic diarrhoea

The (re)emergence of porcine epidemic diarrhoea, caused by a coronavirus, is another example of a disease we thought was well understood, historically being of relatively low economic impact when first discovered in the mid-1970s in the UK [43] (though the effects on young piglets on individual units could be profound) and which could generally be controlled by good biosecurity. Also found to occur in Europe, it was never a significant disease and was rarely recorded, usually as a cause of diarrhoea in adult pigs. It's emergence in Asia in the early to mid 1980s as significant pathogen, infecting all ages of pigs but causing high mortality in neonatal piglets was unexpected, as was its appearance in the United States in the early 2013, which had previously been free of the disease. It rapidly spread across the US, causing

significant economic loss and the deaths of more than 8 million newborn piglets in that country during a 1 year-epidemic, with onward spread to Mexico and Canada [44, 45]. There is also genetic evidence of spread from the US eastwards, to Japan, South Korea and Taiwan, China [45]. Spread from pig to pig and farm to farm is by standard pathways of pigs and fomites, with modes of long-distance spread involving spray-dried plasma and blood stimulating much debate over the significance of that pathway. Semen has also been recently been implicated as an additional potential pathway.

Retrospective genetic analysis has shown that these early strains of PEDV from the late 1970s and 1980s were almost identical from the "classic" virus described at the time of its discovery. In Asia, the first reports of significant disease appeared in Japan in 1982, with severe epidemics involving high piglet mortalities reported since that time in a number of Asian countries.

In the early 1990s, the launch of an inactivated vaccine, based on the prototype CV777 strain of PED seemed to largely control the disease for some years. However, in the mid-2000s, a variant of PED seems to have emerged, designated as G1b, to differentiate it from the "classic" PEDV G1a), which was first detected in China and from 2010-2014 in parts of Europe [46].

In 2010, there was a significant increase in cases of PED in China, sometimes in in vaccinated herds, with new variants of PEDV being implicated [47]. This strain has since diversified and spread throughout Asia and analysis shows two new, related subtypes of a new genotype, G2a and G2b [46]. So, where did this "novel" strain, or strains of PEDV come from? The short answer is that we don't know, but we do know that, as RNA viruses with multiple rounds RNA to RNA transcription, coronaviruses also have the ability to generate diverse quasispecies, albeit perhaps not so divers as the related arteriviruses, since coronaviruses do have some transcriptional error correction capability.

Molecular biologists as an "evolutionary driver"?

Finally, I would like to highlight a rather new (at least in evolutionary terms) driver of viral evolution – and that is those of us who work in laboratories, engineering viruses in our efforts to understand pathogenicity and attenuation, correlates of protection and in development of new vaccines. The ethical responsibilities of scientists and the requirement to assess the risks of their work and take proportionate mitigating steps, are well documented [48]. But it is a sad fact that there is insufficient scrutiny in many parts of the world, with the inherent risks of engineered viruses escaping from laboratories and novel, engineered vaccines reverting to virulence and causing disease becoming all too real. It is not really possible to say at this time whether this is a significant risk, but given the huge advances being made in cloning and the increasing accessibility of such technologies, I would urge utmost rigour in risk-benefit analysis as part of such work and application of stringent biosecurity and biosafety standards where any risks are identified and work proceeds.

Conclusions

I hope I have provided sufficient evidence to show how viruses adapt to changes in their environment, that the global intensification of pig farming and associated international movements can influence their pathogenicity and have likely contributed to their ever-increasing diversity. In particular, evolutionary benefits can accrue to RNA viruses due to the nature of their replication, which can lead to improved relative fitness in their environment. Our current solutions to the challenge of virus emergence and re-emergence in high density husbandry systems is primarily by development of prophylactic vaccines to reduce the susceptibility of pigs, along with biosecurity barriers at farm and international level to reduce the risk of spread. But we know that prevention is impossible – we can, at best, only reduce such risk.

Perhaps a better approach would be to understand more of the molecular basis of viral evolution and develop methods to intervene. Herein, I have tiptoed through the complex world of virus evolution and quasispecies theory. Evolutionary biomathematicians have themselves now moved on to headier areas of debate, including whether one can impair viral replication by intervening in the interactions of mutant spectra, so inducing information meltdown – error catastrophe. This is essentially driving the relative fitness to zero. If this can be achieved, it offers the exciting prospect of a whole new field of vaccine design, which targets the replicating virus directly, rather than via the immune system of the pig.

But until such theories become reality, we must all learn to appreciate the dangers inherent within large-scale pig production and international trade in pigs, pig products and germplasm. We must understand that the high density and

almost clonal nature of pig genetics can provide a "monoculture" environment which may select out highly pathogenic viral clades, leading to explosive outbreaks of novel disease. This drives an ever-increasing demand for new vaccines, which, in this age of molecular engineering, may themselves potentially pose a threat. Also, this dual "pathogen and prophylactic" challenge to the young piglet can be formidable and it is debatable whether it can be sustained.

It is the nature of evolution that the fittest within a given environment proliferate, so it is logical that, if such proliferation is encountered, then it is the environment that is the driver. The pig industry, the veterinarians and scientists which serve it and the international agencies that police it, all need to take a step back and re-evaluate the key drivers behind the many novel disease problems being encountered within pig production and the soundness of the current scientific approaches being applied to solve them.

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Understanding and combating pig proliferative enteropathy: Any news from the frontline?

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Keywords: pig proliferative enteropathy, *lawsonia intracellularis*, genomics, pathogenesis

Porcine proliferative enteropathy (PPE) is a transmissible disease caused by the intracellular bacterium *L. intracellularis* (LI). PPE is a problem in swine production systems worldwide. In weaner and grower pigs, mild clinical signs, also known as proliferative intestinal adenomatosis (PIA), consist of non-haemorrhagic diarrhoea, ill-thrift characterised by macroscopically obvious thickening of the intestinal mucosal lining which is due to proliferation of epithelial cells which line intestinal crypts, mainly in, although not restricted to, the distal ileum. There is a consistent close association between this proliferation and the intra-cytoplasmic presence of LI in the hyperplastic crypt epithelial cells. The PIA form of the disease is resolved 7-8 weeks from the start of the infection resulting in pigs that are underweight prior to entering the grower-finisher stage. In adult pigs, the infection can lead to an acute haemorrhagic form of the disease, also known as proliferative haemorrhagic enteropathy (PHE), escalating from bloody to dark, tarry diarrhoea which may result in death. Sub-clinically cases of infected pigs without clear clinical signs also exist and result in reduced growth performance. Further, some have reported that pigs may shed LI intermittently over a prolonged period of time (up to 8–12 weeks).

This talk will offer the opportunity to review briefly the background of PPE disease and its transmission and control and will describe recent advances regarding LI genomics and its pathogenesis. Finally because PPE still represents a challenge for the pig industry and yet little is known about the potential “modus operandi” of LI to cause the pathogenesis I will discuss novel ways forward to further enhance our understanding of the disease which may help tackle PPE more vigorously.

This talk is dedicated to the memory of Dr Gordon Lawson who died in January 2018, He was the head of veterinary pathology at the Royal (Dick) School of Veterinary Studies at the University of Edinburgh until 1996. Dr Lawson and his team of PhD students were responsible for the painstaking development of tissue culture techniques that enabled growth of LI bacteria, and later successful reproduction of PPE disease.

Pathogenicity of *Haemophilus parasuis and* control of Glässer's disease

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Haemophilus parasuis is a member of the nasal microbiota of healthy piglets. However, under certain circumstances some strains of *H. parasuis* can disseminate systemically and cause Glässer's disease, which is characterized by fibrinous polyserositis. Isolates of *H. parasuis* are very heterogeneous and differ in genotypic and phenotypic features, including pathogenic capacity. This heterogeneity, together with the fact that several strains can be isolated from a single animal and from a given farm, create a complex epidemiological situation that needs to be analysed for a precise diagnosis and an effective control of disease.

H. parasuis is an early colonizer of the upper respiratory tract of piglets and it can be found in basically all commercial farms. However, Glässer's disease occurs only in some farms. The major risk factors for disease development are the suboptimal management of the animals, lack of specific immunity, co-infections and virulence of the *H. parasuis* strains in the farm. *H. parasuis* starts the process of infection by colonizing the upper respiratory tract. This step of initial colonization is common to virulent and non-virulent strains and it is probably promoted by bacterial adhesins. After the initial colonization of the upper respiratory tract, virulent *H. parasuis* strains can be found in the lung of piglets, since they survive the phagocytosis by alveolar macrophages. On the other hand, non-virulent strains are cleared from the lung through phagocytosis by alveolar macrophages, and consequently are retained in the nasal cavity. During lung infection, virulent strains induce a delay in the activation of alveolar macrophages that, together with the adaptation of the bacterial metabolism and the expression of virulence factors, promote bacterial survival in the lung. From the respiratory tract, virulent strains can spread systemically and induce the strong inflammation characteristic of Glässer's disease. Systemic infection is possible by the resistance of virulent strains of *H. parasuis* to serum complement, and their ability to adhere and invade epithelial and endothelial cells. However, the virulence factors of *H. parasuis* are not totally characterized.

Like in other bacterial infections, antibiotics are commonly used in the control of Glässer's disease. Glässer's disease affects mainly nursery piglets and in many farms perinatal antibiotics are used for control of this disease. Since the sow herd is the main source of the pathogen, antibiotics in sows to reduce transfer of the bacterium to the piglets are sometimes also used. Currently, public and private institutions are pushing towards a reduction in antibiotic use due to the emergence of drug resistances, which is a major health problem. An additional problem of antibiotic treatments is that many drugs do not specifically target pathogens (in this case the virulent strains), but also affect the beneficial bacterial communities of the microbiota. Nasal microbiota composition has been recently shown to be a predisposing factor in Glässer's disease development, opening new possibilities of promoting health by manipulating the microbiota composition. More specific control strategies, such as vaccination to eliminate virulent strains, constitute also promising alternatives. The role of antibodies in protection against Glässer's disease has been demonstrated and can be explained, at least partially, by their capacity to opsonize virulent strains and render them susceptible to phagocytosis.

Genomic comparisons of *H. parasuis* strains showed that the virulence-associated trimeric autotransporters genes (*vtaA*) were differentially present in strains from different clinical origins. Two of these proteins, VtaA8 and VtaA9, have been shown to be involved in phagocytosis resistance. Additionally, and in agreement with their role in virulence, expression of genes of the *vtaA* family are induced during lung infection. Analysis of the sequence of these genes in different strains has allowed the development of a PCR for the identification of potentially virulent strains, but more importantly, a combination of VtaAs was shown to be a good vaccine candidate. The opsonic monoclonal antibody 69C6 produced against VtaA8 allowed the identification of a surface exposed epitope in the C terminus of the passenger domain of the VtaAs associated with virulent strains. Induction of antibodies against the 69C6 epitope by vaccination would allow the specific target of virulent *H. parasuis* strains.

Keywords: *haemophilus parasuis*, Glässer's disease, strain variability, bacterial pathogenicity, vaccine

Pathogenesis, diagnosis and control of classical swine fever virus and other porcine pestiviruses

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Classical swine fever (CSF) is one of the most important viral diseases in pigs worldwide. In many parts of the world great efforts are being undertaken to reduce economic losses caused by CSF or to eradicate the disease. Among the member states of the European Union (EU) a harmonized strategy for diagnosis, control and eradication of CSF has been applied. Success of the common strategy is documented by the decreasing number of outbreaks during the last decade resulting in the absence of CSF in all EU member states. Nevertheless, CSF remains a continuous threat to the European pig and wild boar population. Recent activities of the EU & OIE Reference Laboratory for CSF will be summarized. These activities include (i) organization and quality assurance of CSF diagnosis in the EU and development of novel diagnostic tools, (ii) update of the CSFV sequence database, and (iii) identification of new pestiviruses and differentiation from CSFV.

In addition to CSFV, other pestiviruses can infect pigs. In contrast to rare or even unique infections of pigs with BVDV, BDV, Bungowannah virus and Linda virus, the recently discovered, only distantly related atypical porcine pestivirus (APPV) is widely distributed in domestic pigs of Europe, Asia as well as North and South America and represents a major cause of congenital tremor in newborn piglets. After successful establishment of assays for detection of APPV genomes and APPV-specific antibodies, course of infection, tissue tropism, presence in wild boar and putative cross-reactivity of CSFV and APPV infections were studied and may contribute to the establishment of strategies to control APPV infections.

Gene edited pigs are resistant to PRRSV infection whilst maintaining biological function of the editing target gene CD163

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Porcine reproductive and respiratory syndrome (PRRS) is a panzootic infectious disease of pigs, causing major economic losses to the world-wide pig industry. PRRS manifests in pigs of all ages but primarily causes late-term abortions and stillbirths in sows and respiratory disease in piglets. The causative agent of the disease is the positive-strand RNA PRRS virus (PRRSV).

PRRSV has a narrow host cell tropism, limited to cells of the monocyte/macrophage lineage. CD163 is expressed at high levels on the surface of specific macrophage types and soluble protein is circulating in blood. CD163 has been described as a fusion receptor for PRRSV, with the scavenger receptor cysteine-rich domain 5 (SRCR5) region having been shown to be the interaction site for the virus.

We have generated pigs in which Exon 7 of the CD163 gene has been deleted using CRISPR/Cas9 editing in pig zygotes. These pigs express CD163 protein lacking SRCR5 (ΔSRCR5 CD163) and show no adverse effects when maintained under standard husbandry conditions. The ΔSRCR5 CD163 was not only detected on the surface of macrophage subsets, but the secreted, soluble protein can also be detected in the serum of the edited pigs. Macrophages from these animals are resistant to PRRSV-1, subtypes 1, 2, and 3, as well as PRRSV-2 infection *in vitro*. An *in vivo* challenge experiment, with ΔSRCR5 pigs showing resistance to PRRSV-1, subtype 2 infection, confirms our previous *in vitro* data.

This research shows that genome editing opens new opportunities for next-generation breeding for virus-resistance in livestock and eradication of disease. It highlights the need to study host-pathogen interaction on a cell-biological and structural level for veterinary viral pathogens. Understanding these interactions will allow the most subtle changes to host proteins to achieve virus-resistance whilst maintaining biological function of the protein.

Manure treatment and utilization in China

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With development of intensive animal industry in China, environmental pollution caused by manure of 3.8 billion tons per year is becoming more and more serious. To control manure pollution and promote the green production of animal industry, related regulations or strategy related were promulgated, and seven typical manure utilization modes were recommended by Ministry of Agriculture and Rural Affairs of China, Including mode 1 whole manure collection and land application, mode 2 specialized energy production, mode 3 solid manure composting, mode 4 ectopic manure fermentation bed, mode 5 litter recycling, mode 6 recycling cow dung as bedding materials, mode 7 up-to-standard discharge of waste water. Given the applicability of different mode and diversity of ecological and meteorological conditions, as well as broadest spectrum of elements in animal manure and waste water, special manure mode need to be identified for different livestock and poultry farms. It is worth mentioning that the ideas of waste treatment and utilization is consistent, such as, source reduction, process control, and end use to achieving a win-win environment and the economy. During utilization process, the key point is estimation of land carrying capacity. Comprehensive nutrient management planning can be used to evaluate the land application, to ensure manure application scientifically. More remarkable, the necessary manure treatment and utilization process to mitigate antibiotics in urine and feces need, such as anaerobic digestion, composting need to be considered in manure management system.

Keywords: manure treatment, utilization, mode, and nutrient management, antibiotic.

Novel mechanisms of PRRSV infection: intercellular transmission and persistence

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Porcine reproductive and respiratory syndrome virus (PRRSV) infection can be divided into at least two distinct stages: acute infection and persistence. Initial acute infection leads to the cytopathic replication of the virus in host cells, resulting in the release of large amounts of viral particles that subsequently transmit to naive cells through standard clathrin-mediated endocytosis. This process needs abundant cellular resources and also requires that the viral particles evade extracellular host defensive components. Recently, alternative pathways for intercellular spreading of PRRSV infection have been identified, in which viral infectious materials can be transmitted through intercellular nanotube connections (TNTs) and exosomes. Utilizing these pathways to directly access the cytoplasm of a naive cell present efficient spreading routes for the virus to bypasses many of the otherwise critical assembly, budding, and cell entry steps. Mitochondrion, an important regulator for cell survival and cell death, appears to be transported through TNTs to regulate the fate of PRRSV-infected cells. This pathway is currently being explored as a therapeutic strategy. On the other hand, PRRSV takes advantage of this pathway and utilizes the mitochondrion as a cargo to transport viral infectious materials for cell-to-cell spreading of the infection. Intercellular transmission through these novel pathways allows the virus to escape the host immune responses, which may contribute to the pathogenesis of viral infection and persistence.

Current advanced technologies have enhanced our capability for in depth studies on the molecular mechanisms of PRRSV persistence. A cellular model of a persistently infected cell line has been established. Using this model system, viral dsRNA was revealed to be associated with the TNT pathway and function as a mediator for PRRSV persistence. Pig models have been used to confirm the findings in the cell culture system. PRRSV dsRNA was detected persisting in lymphoid tissues of infected pigs. Importantly, the germinal center of the lymphoid tissue was identified as a potential reservoir for dsRNA persistence. A swine immune gene specific RNA array was developed to further study the interaction of dsRNA persistence and host immune responses. The RNA array analysis showed that dsRNA in lymphoid tissues of persistently infected pigs had limited ability to stimulate host antiviral responses, suggesting that viral dsRNA persistence in the germinal center allows the virus to escape antiviral immune responses. The array analysis further revealed the potential immune gene signaling pathways involved in regulating PRRSV acute/persistent infection. Studies are underway to identify the potential pathways and specific cellular genes as indicators for viral persistence.

The ability of PRRSV to invade the host immune system and establish persistent infection significantly impedes our efforts to eliminate PRRS. Novel mechanisms of PRRSV transmission and persistence revealed in our recent studies provide fundamental knowledge and new directions for developing diagnostic assays, therapeutics and other control measures.

Advances in the development of novel marker vaccines against classical swine fever

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Live attenuated vaccines against classical swine fever (CSF) are well known for their good protective efficacy. In countries where CSF is still epidemic, mass immunization with live vaccines is the common practice, while eradication program has been on their agenda. However, the major issue facing CSF eradication is lack of suitable vaccines and accompanying differential testing system to distinguish infected from vaccinated animals (DIVA) by simple serological methods. Considerable progress has been made in the past decade in the development of marker vaccines and companion diagnostic kits.

Several early studies used recombinant DNA technology to produce subunit marker vaccines targeting the highly immunogenic E2 glycoprotein of CSF virus (CSFV). Although E2-subunit marker vaccines could provide clinical protection with reduced viral shedding, there are apparent drawbacks such as lack of early protection and failure to prevent transplacental transmission. An adenovirus-delivered E2 vaccine was shown to induce sterilizing immunity comparable to the C-strain vaccine with the potential of DIVA purpose using an Erns-based ELISA. The use of reverse genetic systems has made it possible to generate live attenuated marker vaccine (LAMV) constructs with different formats. A new LAMV 'Suvaxyn' licensed in Europe in 2014 is a chimeric virus with the bovine viral diarrhea virus (BVDV) type 1 strain CP7_E2alf as backbone that carries E2 of CSFV group 1 strain Alfort/187. The vaccine provides protection at 5-7 days post-immunization with immunity lasting for 6 months. Diagnostic tools are available for both genetic and serological DIVA. Similar approaches were attempted by replacement of Erns or E2 in the BVDV group 2 strain genome with the CSFV counterparts. Another marker vaccine candidate FlagT4Gv was derived from CSFV group 1 strain Brescia with modification of codon usage as well as insertion of Flag epitope and abolition of a conserved CSFV-specific epitope recognized by mAb WH303. FlagT4Gv was shown to retain the attenuated phenotype and conferred effective protection against challenge with virulent CSFV. A mutant virus in the E2 WH303 site of the C-strain backbone is another candidate with DIVA function. Majority of the above studies used E2 fragment of CSFV group 1 strains. Recent studies have shown that the virus populations in the field have switched from group 1 to group 2. It remains unknown if chronic or atypical form of CSF is due to such switch as a result of positive selection with long-term intensive immunization. We found that replacement of C-strain with a hypervariable region (HAR1) of a group 2 virus could induce higher neutralizing titers than its parental C-strain to field viruses. Therefore, we constructed several C-strain based chimeric viruses containing the E2-HAR1 and marker regions and tested their potential of improved efficacy and DIVA function. The recombinant viruses showed better replication than their parental strain.

In summary, promising marker vaccine candidates against CSF are currently available. Further efforts are still required to demonstrate their genetic stability, safety, spectrum against various virus variants as well as sensitivity and specificity of the accompanying DIVA diagnostic tests.

Herd health management through prevention and control of pathogens Prevention starting with biosecurity

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Biosecurity is a top concern in the livestock industry since it affects performance, economic results and can even close markets for trading when catastrophic disease occur. Excellent examples are African Swine Fever (ASF), Classical Swine Fever (CSF), and Foot & Mouth Disease (FMD) that will dramatically affect export sales of a country. Biosecurity in its simplest sense is how one reduces the risk of pathogens from entering the site which is called bioexclusion. Another aspect of biosecurity is called biocontainment which is how one controls the transmission of a pathogen within the production site. Biomanagement is the combined activities of both bioexclusion and biocontainment. All biosecurity programs need to be practical; thereby encouraging implementation of the plans. The most difficult aspect to implementation is ensuring that the individuals performing the daily biosecurity procedures completely understand the importance of their actions. Additionality the individuals need to know that it is a team approach by everyone on the staff and commitment to doing the correct procedures each and every day. The understanding of the procedures is usually easily adopted but the motivation to perform the proper procedures each and every instance can be lacking at times. Every employee must embrace all aspects of the biosecurity program for successful implementation.

In years past biosecurity programs were approached by an organized educational sessions similar to a classroom approach. A disease break creates the need for all employees to immediately assist in the necessary activities. The new disease break creates a "crash course" which increases awareness and seriousness of the situation. The "crash course" methodology does not generate alignment through all departments of the farm's staff or a system-wide sustainable program on biosecurity. Any biosecurity program begins with fundamental practices, plus an attitude of continuous learning as new information develops.

The complexity of small farms has given way to far more multifaceted programs on larger farms. The entire array of potential threats has also been heightened due to multiple strains of a pathogen such as, Influenza type A Virus, where more than one strain commonly persists in a large population at the same time.[1] The variation of pathogenicity is another factor that often creates increased economic damage once it enters a site (PRRSV is a great example of this variability across strains). Today management level employees are skilled and knowledgeable about the following: an awareness of all current processes on the site that can impact biomanagement; understanding the economic harm that occurs when health of the site changes; knowledge on how to reduce risk to the farm; and, having open communications for questions and instructions for all employees.

The different levels of bioexclusion: developing the clean – dirty line

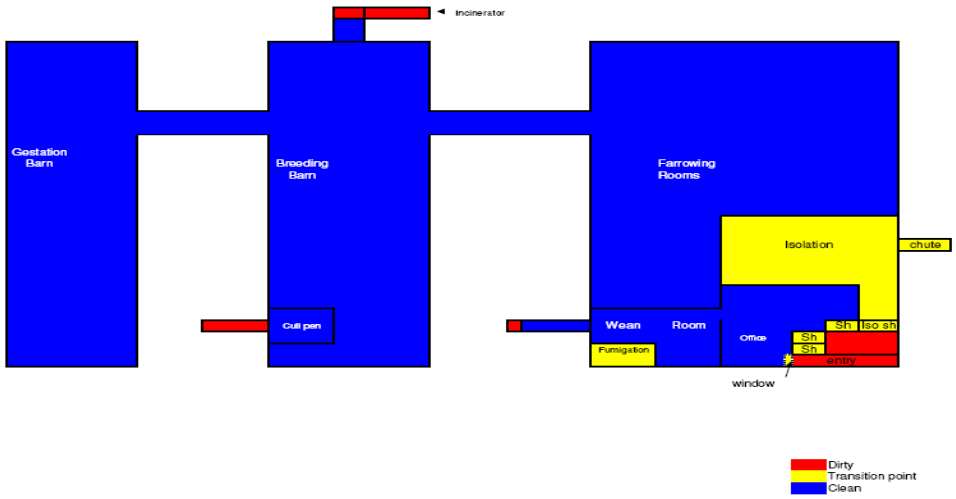
The Secure Pork Supply (SPS) Continuity of Business Plan in the US was developed to provide opportunities to voluntarily prepare before an outbreak like FMD, CSF, and ASF.[2] By participating in the SPS program the unit establishes a premise where the animals are not exhibiting or infected with a foreign animal disease. This allows the unit to:

- Move animals to processing or another pork production premises under a movement permit issued by Regulatory Officials, and
- Maintain business continuity for the swine unit enrolled into the SPS program, including producers, haulers, and packers during an FMD, CSF, and ASF outbreak.

The participation in SPS establishes different levels of bioexclusion starting with a perimeter fence that includes an entry gate. The fence or first layer is meant to make a statement about passing through or beyond this point requires that one needs permission before entering. This can be considered the first layer in developing a clean – dirty line. This layer is used globally as observed by the author. There are variations with a double wire fence of different heights to a solid

concrete wall. The entry gate also varies on the complexity for both vehicle and people entering. The obvious goal is to reduce entry to only those that need to come inside which is the major reason for a clean – dirty line in any biosecurity program.

The second layer of the clean – dirty line is the point of entering the structure for supplies, people, animals, and everything that is needed inside the unit. This clean – dirty line is illustrated by using different colors. The structure's shape is color coded by using red color to illustrate dirty and blue illustrating clean areas of the structure. The yellow color is used to illustrate a transition from dirty into the clean area. Some units have chosen to implement different levels even with the entry process. The simple approach is to enter into the unit with only a clothing change. Most units will have people enter with a complete clothing change, shower and put on clean clothes before entering. A few units have added a bench entry system plus the clothing change, shower and the use of clothes that stay on the clean side. The physical bench is the reminder for the person entering that a "change" is about to occur. A properly managed clean – dirty line within all structures is lacking in units throughout the world. A clean – dirty line for entering any unit needs to be correctly administered to provide further "insurance" against pathogen entry into modern units.



Other potential vectors and additional layers for bioexclusion

The list of what comes into a modern swine unit is daunting when one begins to write everything down. Very simplistically, try the exercise of listing everything that enters a unit sometime. One needs to begin with the obvious things on the list that enters by underground, surface and airborne routes. The list will include necessary objects like water, feed, trucks, trailers, people, mobile phones, supplies of all sorts which includes vaccines, bottles of antibiotics, equipment, bedding material, bags of drying dust, food items for employees, and many more. porcine epidemic virus (PEDV) outbreaks in 2013 - 2014 in North America caused a massive upscaling of biosecurity programs especially for sow farms. Creep feed quickly became a focus for improved control, requiring a minimum two weeks prior to being used in farrowing rooms for example. Recent research has shown extended infectivity of some viruses for up to 37 days with the help of plant products like soy proteins.[3] Bio-shed (a small structure for housing supplies, semen, etc.) were purchased or constructed and placed near the road so non-essential vehicles did not need to enter as far as the unit. Bio-shed management requires a person from inside the unit to exit through the shower and put outside clothing on to collect the supplies. This person then re-enters the unit by showering and using the unit's clothes again. Fumigation rooms were another addition. Supplies, equipment, tools, bags of products, boxes are placed on shelves within the fumigation room. Fumigation rooms were constructed with one outside door for the outside person to unload the supplies into the racks within the fumigation

room. The boxes and items were allowed to set for an adequate period of time before someone inside the unit was allowed to enter and unpackaged the products.

Potential vectors influencing biocontainment: people transmit pathogens around a unit

Several years ago a study was conducted to look at how people transmitted hemolytic *E. coli* around a unit.[4] In this study, people mechanically transmitted *E. coli* without extraordinary measures to enhance caretaker contact with pig excretions and secretions beyond that which would occur in a typical pork production unit. Hand washing and donning clean outerwear did not prevent *E. coli* transmission. However, showering and donning clean outerwear did prevent transmission.

The author's understanding of people transmitting pathogens promoted the use of another aspect of the clean – dirty line when performing partial depopulations in the early days of PRRSV elimination programs. Implementation of a clothing and boot change with sanitizing of the hands, i.e. a clean – dirty line, was established in the hallway of the finishers. The clean – dirty line in this situation is to separate the PRRSV infected rooms from the rooms containing non-infected animals. The nursery is depopulated after establishing sow herd stability as defined by American Association of Swine Veterinarians (AASV) PRRS categories. Infected animals were separated from non-infected animals by an empty finisher room in-between the rooms housing infected and non-infected animals with a temporary curtain in the hallway. A complete change of outer clothes, gloves and boots with a disinfectant containing boot dip pan was made as one walked through the curtain. The curtain was moved down the hallway as the next infected finisher was marketed, cleaned and disinfected. This allowed the empty finisher to be filled with non-infected animals with a cleaned disinfected room maintained in-between.

A summary of a proper entry program for people with the use of a clean – dirty line includes at least the following considerations:

- Comply with "days" away from pigs not associated with the unit you are entering. Example: one night after being in a sow unit with a shower, complete change of clothes, and re-showering into the nursery – finisher is sufficient. A two night time period away from one unit before going into a completely different unit with showers and clothing changes to add sufficient security to not transmit a pathogen between units.
- Leave personal effects, including digital mobile devices in a secure area or your vehicle.
- Walk through disinfectant sprays which is common in Asian countries.
- Remove footwear and all clothing in an outer changing room, leave clothing in the outer room, pass through the shower and put on unit provided clothing. The past thorough shower is another example of the clean – dirty line.
- Thoroughly shower and wash hair using soap and shampoo. The exception to the shower is the entry into down flow sites from the sow unit, i.e. nursery, wean-to-finisher or finisher sites that sometimes only require a bench entry system, i.e. complete clothing change, washing of the hands and using the unit's boots.
- Dry yourself and dress in unit provided clothing and footwear.
- Sanitize hands with alcohol or disinfectant gel.

To ensure compliance of your staff requiring a shower, the facilities must provide:

- ✓ A shower that is kept clean and mold free.
- ✓ A good ambient air temperature for the people during the shower.
- ✓ Plenty of hot water and a shower head with a generous spray.
- ✓ People friendly soaps and shampoos.
- ✓ Clean towels.
- ✓ Clean clothing available at all times to properly fit staff and visitors

Other biocontainment aspects for consideration

Additional efforts are being implemented to control insects within the unit since studies have shown transmission by insect or arthropod as a fomite, i.e. mosquito, different species of flies and cockroaches.[5,6,7] Rodent control has been encourage with the same consideration of being either a fomite or vector for transmission of pathogens around a unit. The author's observations support rodent control measures due to a continual *Lawsonia intracellularis* outbreaks when mice populations are high. *Brachyspira hampsonii* infections have been found in wild water birds and speculated as a

possible source of infecting a naïve farm when the water birds used the unit's lagoon for an extended stay.[8]

Animal monitoring and transportation biosecurity

The most obvious method of bringing a pathogen into a unit is through the animals themselves. Routine profiling of the replacement animals has historically been serology, i.e. blood collected from a random number of animals. The more recent use of oral fluid samples have provided additional knowledge on the population and shedding. The behavior of the pig is such that whatever is in the environment of the animal is in the animal's mouth. Saliva samples have enlightened the industry on persistence of Influenza virus in a population long after the original break.[9] Even more recent work with *Mycoplasma hyopneumoniae* elimination program have driven the use of laryngeal and or tracheal swabs since the serological samples have presented a delay in the animals immune response.[10] The multiplier level units in North America are now using both serology and laryngeal swabs to monitor the replacement animals to protect sow farms that are naïve to *Mycoplasma*.

The activities of monitoring of animals prior to movement, such as replacement animals to be placed into a sow unit, has dramatically changed in the past couple of years. The implementation of using oral fluid samples, i.e. ropes, has enlightened the industry on shedding of pathogens. The sample collection itself has increased the number of animals being monitored since the rope is tied so two pens of animals can chew on the rope. Thereby, expanding the number of animals monitored from when 30 were serologically profiled to two pens has improved detection of pathogens. The goal is to match health or provide replacement animals with better health then the sow population. Recently laryngeal swabbing of replacement gilts to determine *Mycoplasma hyopneumoniae* infection has been driven by the need to detect infection more quickly than the use of serology. The activities of monitoring a population will continue to evolve as better understanding of transmission of pathogens become recognized.

Another method of transmitting disease is with dirty trucks and trailers. Numerous studies have shown and provided knowledge on what it means to have a clean trailer. The author asked a local trucking firm what percentage of producers asked and paid for a clean truck and trailer before porcine epidemic disease virus (PEDV) challenge vs after the massive spread across the hog producing states. The percentage prior to 2014 outbreaks of PEDV was estimated to be around 50% to 60% of the producers wanting a clean truck and trailer. The percentage quickly moved to 95+% of the time producers wanted a clean truck and trailer. A key study suggested that collection points, such as harvest facilities and livestock auction markets, can be an efficient source of contamination of transport vehicles that return to pig farms and likely played a role in rapidly disseminating PEDV across vast geographic regions shortly after PEDV was first identified in the United States.[11] This data also suggests that the contamination of transport vehicles leaving the harvest facilities increased as the prevalence of PEDV–positive transport vehicles and virus load coming into the facility increased. Trucking firms have installed modified forms of "thermal assisted drying" by blowing heat into the trailer after a proper cleaning and disinfecting. Although this additional practice is not entirely necessary, the addition of heat has added value of providing a clean trailer to the producer. The following list was a combined effort of American Association of Swine Veterinarians (AASV) and National Pork Producers Council (NPPC) personnel to provide proper instructions for both producers and truckers to prevent pathogen entry into the unit. A form of the clean – dirty line is also illustrated in these instructions since live animals cross over this "line" when entering the transport trailer but people do not cross.

Ensure you are Prepared for Swine Transportation

1. The market truck must be prepared for hauling market hogs.

a. The cab of the truck, including floor-boards, pedals, steering wheel, gear shift handle, door handles, etc., must be cleaned and disinfected between loads.

b. The trailer must be

• Washed clean and free of any visible manure or shavings,

• Disinfected with an appropriate disinfectant, at the correct rate, for the proper contact time, and applied so that all surfaces are covered, and

• Allowed to dry completely (Thermal assisted drying speed this process greatly).

c. All equipment, including sort-boards, rattle paddles, electric prods, etc. need to be thoroughly cleaned, disinfected, and

dried.
d. Clean boots, coveralls, gloves, knee pads, etc. must be used for each load and stored in a designated clean area. A separate area for storage must be used for these articles after they are used and until they can be cleaned and disinfected for future use.
2. The Production Site must be ready for animal movement.
a. All load out equipment must be clean and in good working order
b. The load out area and chute must be clean, disinfected and ready to market pigs
c. Communicate where the clean – dirty line is located. This marks the separation between the production facilities, its animals and its workers from trucks, trailers and people outside of the production facility.
• An effective clean – dirty line is the back of the trailer but may be at the barn door, the chute or gate.
• Be sure it is clearly marked and visible to all.
• Provide plastic disposable footwear and a place to dispose of the footwear for the driver if they must to cross the clean – dirty line.
d. An amp supply of trained farm personnel available to help load pigs from the site.
• The truck driver should never cross the clean – dirty line to help move pigs from the barn.
3. Communication between the livestock hauler and livestock owner or site manager must be open and complete.
Expectations for loading and unloading animals must be communicated prior to arrival.
a. A clear clean – dirty line must be identified and communicated.
• No human foot traffic is allowed to cross the clean – dirty line from either direction.

Responsibilities for compliance by the different parties during the Loading Process
1. Livestock haulers
a. Must stay on the out-bound side of the clean – dirty line at all times for load out.
b. No driver equipment may cross the clean – dirty line or be used in the barn.
c. No pigs should be allowed to exit the truck to re-enter the unit during the load out process.
d. The driver must remove all boots and clothing on the truck side of the clean – dirty line.
• All dirty boots and coveralls should be placed in a designated area, outside the cab (for example in a dirty boot box).
e. Hand paperwork to farm load-out personnel away from the truck and barn.
2. Loading crew or farm personnel
a. The farm load crew must observe the clean – dirty line at all times.
• If the clean – dirty line is crossed, farm personnel MUST follow re-entry biosecurity measures (such as shower in/out or change of clothes/boots and wash of hands) before they can resume the loading process.
• Dirty coveralls or gloves must be placed in a container or directly into a washer.
• Dirty boots must be placed where they can be washed and disinfected away from farm clothing. Do not place them where everyday foot traffic occurs.
• Do not share loading equipment with livestock haulers.
b. Do not cross foot traffic at any time with livestock haulers including after pigs are loaded.
c. No farm equipment should be shared with the livestock haulers.
d. Do not allow drivers to help load pigs out of the barn.
e. Do not allow drivers to fill out paperwork in the office.

Responsibilities after the Loading Process
1. Farm personnel must clean and disinfect the load out area immediately after the transport vehicle has been loaded and pulled away.
2. Farm personnel that cross the clean – dirty line to clean the chute or load out area must follow the biosecurity protocols of the site, such as shower in/out or change of clothes and boots and wash hands.

Control of a pathogen using Porcine Reproductive and Respiratory Virus (PRRSV) as the example

Porcine reproductive and respiratory syndrome (PRRS) despite decades of intense research and vast amounts of resources, remains the most costly production disease throughout the world. Progress continues to be made in controlling this pathogen, but the estimate that PRRS costs the US swine producers every year is more than US$580 million.[12] The cost to a producer is both in the sow unit and in the nursery finisher flows. Economic losses are significantly larger in naïve herds post-infection of PRRSV then compared to herds that are antibody positive at the time of the PRRSV infection.[13] PRRSV can be consider a "tariff" or a demand on the unit's resources that must be paid as long as the virus is active and causing damage to the production of the animals.

PRRSV continues to find its way into modern swine units even when tremendous resources have been implemented to impede the virus's ability to enter. The majority of PRRSV impact occurs during the acute outbreak especially in naïve populations, but this is also the time when the management can focus on minimizing the death loss of the sows, piglets and growing pigs that became infected during farrowing. A second goal is to minimize the length of time the sow populations shed virus. Two metrics for measuring both goals have been developed to help capture the impact of a PRRSV break.

1. Time to stability (TTS): TTS measure the duration of the piglet infection. The calculation is the number of weeks post-closure of the sow herd (replacement gilts are loaded into the sow herd with no additional entries until after shedding stops) until four consecutive negative serologic results, i.e. usually serum from 30 individual piglets near weaning age and sampled as one piglet per litter, have been documented.
2. Time to baseline production (TTBP): TTBP measures the volume or number of piglets that dies. The calculation is the number of weeks it takes for the sow herd to return to producing the same volume of weaned piglets per week that it averaged prior to the PRRS outbreak.

PRRSV acclimation programs' main focus is to minimize both the duration of infection and the volume of mortality during an acute outbreak. Thereby improving both TTS and TTBP which ultimately reduces the economic impact of the outbreak. Proper acclimatization will not prevent future infection, but prior exposure minimizes clinical disease when wild-type PRRS exposure occurs. All acclimatization programs are similar since the focus is to take a susceptible population to a state of immune competence that minimizes clinical disease. This process is called immune management of that population.

The biomanagement of PRRSV once it has entered a population also impacts the TTS and TTBP. The author has experienced elongated TTS when mistakes by the employees occur. One example is when a unit weaning one time per week, did not have a focus on controlling internal transmission during a PRRSV elimination program. The employees returned to their daily routines immediately after the weaning task was accomplished. The lack of not cleaning and disinfecting the common hallway, not changing clothes, washing hands or changing gloves, and not using disinfectant boot dips carried virus into the recently farrowed piglets. This was determined by serologically profiling the recently farrowed piglets which determined that they were being born naïve to PRRSV. This established that the sow herd was stable and not shedding virus. Several changes were implemented as suggested previously and immediately the near wean pigs went PRRSV negative and stayed negative to present.

Another example is when strict "management changes to reduce exposure to bacteria to eliminate losses" or also called McREBEL is practiced during a PRRSV elimination program.[14] Strict McREBEL mean that no cross fostering is performed. When strict McREBEL is practiced more starve-out piglets will need to be timely euthanized. The act of euthanizing the piglets is again in the common hallway where entry into the farrowing rooms occur. The hallway was not sanitized properly immediately after euthanizing the piglets; therefore, the employees carried the virus into the farrowing rooms with the youngest piglets. The transmission of virus in this manner caused an elongation of the elimination program.

Herd health management with a goal of preventing or at least controlling pathogen activity so economic harm is reduced, first starts with a detailed plan for all departments. This aspect is called the unit's biosecurity program. Bioexclusion is the portion of the unit's plan to keep pathogens out of the facilities. Biocontainment is the portion of the biosecurity program that minimizes or controls the economic damage from the infection. Biomanagement is both bioexclusion and biocontainment working together to achieve the unit's goals on pathogen prevention and or control. One approach is to have routine staff meetings where biosecurity is discussed. The following discussion topics can be used as a guideline:

1. List and define all biosecurity practices by department.
2. Develop biosecurity gap or "hole" in each department.
3. Define solutions for each respective gap.
4. Empower one person in each department or at least one person per unit to be the champion of biosecurity who looks at each practice to determine is a change is needed or if the practice is sufficient.
5. Build interdepartmental relationships so alignment and collaboration occurs for the overall health programs.

At the end of the day biosecurity is never stagnant but a dynamic ongoing program that needs monitored and often discussed. The science on pathogen transmission is ever evolving which causes the person who is the champion of biosecurity for each unit to re-examine the programs. The key is to develop a culture for the employees that empowers them to come along side and work together for the best health of the unit.

The author would like to thank numerous individuals for challenging and educating him on biosecurity by participating in exercises and meetings illustrating the need to improve current traditions. Also the author appreciates each and every producer that has worked with him thorough a health challenge. These experiences have provided insights into how to improve upon current biosecurity practices.

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Actinobacillus pleuropneumoniae: why do we still have problems to control the disease?

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Porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae* (App) is a contagious respiratory disease, reported to cause economic losses worldwide. Clinical disease in its outbreak presentation is partially well controlled in USA/Canada, but is still a serious problem, sometimes with high mortalities rates, in many Latin-American, Caribbean, Asian and European countries. The disease can also take a chronic form with less mortality, although production losses could still be suffered and lesions at slaughter (adherence, pleuritis and lung abscesses) are usually observed. Finally, App is present in a sub-clinical form in many herds, where animals harbour the pathogen in tonsillar crypts, becoming a source of infection for naïve subpopulations. So far, there are 18 described serotypes; high and low virulent strains exist, usually associated to a given serotype within the same country/region.

Although swine pleuropneumonia is considered an "old disease", there are many aspects of the infection that are yet not well understood and many contradictions are observed in the field. Virulent App strains can cause important mortality in a farm even in the absence of predisposing factors/diseases. In such situations, it may be considered as a primary agent of respiratory disease. However, some farms infected with the same strain may, for unknown reasons, remain free of clinical pleuropneumonia. In addition, lower virulence strains may significantly enhance its pathogenic potential in the presence of concomitant factors, although this is not systematically observed. Infectious (SIV, Aujeszky virus, and/or *Mycoplasma hyopneumoniae*) as well as non-infectious factors, such as crowding and adverse environmental conditions with rapid changes in temperature and high relative humidity coupled with insufficient ventilation, mixing animals from different origins and lack of AI-AO management, may promote the development and spread of the disease and, consequently, affect morbidity and mortality.

Although good vaccines are available in the market to help controlling mortality/morbidity, they should be appropriately used. Even the best vaccine will not prevent disease if other aspects of the infection are not under control.

In this presentation, the main causes leading to the expression of clinical pleuropneumonia and/or chronic disease will be presented. Methods to control and prevent disease will also be discussed.

Key words: swine pleuropneumoniae; prevention; control; carrier animals

A decade journey with the Chinese HP-PRRSV: what have we learned about its pathogenic mechanisms?

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Emergence of the Chinese highly pathogenic PRRSV (HP-PRRSV) represents a milestone along the course of PRRSV evolution. Its sudden appearance in 2006 in mainland China and then subsequent quick spread to major swine farms within the country and surrounding regions caused colossal economic losses to Asian swine industry. Ever since, HP-PRRSV has remained the dominant genotype of PRRSV circulating in the Chinese swine farms despite the increasing incidences of NADC30-like strains or the recombinant derivatives between the two in recent years.

The Chinese HP-PRRSV causes clinical symptoms that are different from that by classical strains, and the key clinical features of infections are marked by high fever (41-42°C), high contagiousness, and high morbidity and fatality (up to 100%). Multifocal hemorrhages are the most notable gross lesion that involve multiple tissues or organs, including skin, lungs, lymph nodes, kidney, and heart. The second is lymphadenopathy and markedly elevated interstitial pneumonia reflected by severe pulmonary edema and consolidation. The third type of lesions is severe thymus atrophy that was often observed in HP-PRRSV-infected piglets. Finally, bacterial secondary infections are a common problem associated with HP-PRRSV infections. The synergistic effect of coinfections potentiates HP-PRRSV induced-pathogenesis and is now a subject under intensive investigations.

12 years have passed since the initial outbreak, and the collective efforts by the Chinese PRRSV research community and others have enabled us to come to understand more about this deadly pathogen with respect to its basic pathogenic properties, the virulence determinants and immunosuppressive aspects. The mechanistic details are now starting to emerge, as key viral proteins are continuously being identified that contribute to the differential regulation of host immunity and viral replication, and new mechanisms are increasingly being found of how HP-PRRSV highjacks the cellular pathways to promote viral replication. The key findings are summarized below.

(i) **Expanded tissue tropism**. HP-PRRSV clearly has gained the ability to invade multiple tissues or organs by targeting macrophages of lungs and lymphoid organs as well as epithelial and endothelial cells from a variety of tissues. This finding suggests a critical mechanism for the high pathogenicity of HP-PRRSV.

(ii) **Enhanced ability to induce cell apoptosis**. HP-PRRSV induces thymus atrophy of piglets, leading to apoptotic cell death of thymic T cells. This likely has a detrimental effect on the development of naive T cells, and potentially affects the nature and quality of newly developed T cell existing in the thymus, predisposing piglets to a weak cellular immunity. In addition, the increased apoptotic cell death of macrophages can dampen the clearance of bacterial pathogens in the lungs, promoting bacterial secondary infections.

(iii) **Aberrant inflammatory cytokine responses**. HP-PRRSV induces overproduction of proinflammatory cytokines (e.g., IL1, IL-6 and TNF-α) that can cause acute injuries of lungs; the persistence of high levels of the proinflammatory cytokines likely has an adverse effect on the body, leading to the failure of multiple organs. In addition, the up-regulation of IL-10 and TGF-β can lead to skewed Th1 responses. Moreover, several viral proteins have been identified that confer the Chinese HP-PPRSV strains an advantage over low pathogenic PRRSV strains in shaping the innate responses, and these include nsp1β, nsp2, nsp4 and nsp11.

(iv) **Predisposition to bacterial infections and pathogenesis potentiation by bacterial infections.** HP-PRRSV creates an environment that is conducive to bacterial infections. These include but not limited to impairing microphage function, inducing thymus atrophy, reducing the number of $CD4^+$ Th17 cells locally and in circulation. On the other hand,

several studies have shown that HP-PRRSV and bacteria (or components such as LPS) can act in synergy to activate inflammasomes.

(v) **Dysregulation of host immunity.** Immune evasion is a general property of PRRSV, but HP-PRRSV possesses an increased ability to down-regulate the MHCI expression on the cell surface of macrophages, an event that is mediated by nsp1α and nsp4. In addition, many nsps and several structural proteins have been found to interfere with the interferon signaling. Thus, HP-PRRSV generally induces weak interferon responses, and a delayed, low-level cellular immunity and neutralizing antibody production. Moreover, the neutralizing antibodies are strain-specific and often lack cross-neutralizing ability.

(vi) **RNA polymerase, viral evolution, replication and virulence.** The error-prone nature and recombinative property of nsp9 are responsible for the rapid evolution and diversity of PRRSV strains that lead to emergence of numerous virulent PRRSV strains in the field. Recently, nsp9 (RdRp) and nsp10 (helicase) have been shown to be the key viral determinants for the fatal virulence of HP-PRRSV, and the key amino acids within nsp9 have also been mapped out. Although how nsp9 and nsp10 exactly contribute to the virulence is not clear, but the low pathogenic virus carrying HP-PRRSV nsp9 and nsp10 have increased replication efficiency in primary porcine alveolar macrophages, while the HP-HPRRSV carrying nsp9 and nsp10 from low pathogenic virus has decreased replication ability, suggesting that viral propagation efficiency may be a key factor to viral virulence. Compared with LP-PRRSV or attenuated vaccine strains, it is commonly found that more virulent PRRSV field strains, such as Chinese HP-PRRSV can grow to significantly higher levels in pigs, exhibiting longer and more elevated levels of viremia and higher viral loads in tissues, as well as inducing faster and more intense humoral immune responses and more severe clinical signs in inoculated animals. As well, the attenuated strains only showed a limited number of replication cycles. This feature may in part account for the increased pathogenicity and fatal virulence of the Chinese HP-PRRSV for piglets. The mechanisms of how the mutations in nsp9 and nsp10 synergistically promote HP-PRRSV replication both *in vitro* and *in vivo* remain poorly understood, and should be addressed in the near future.

Future perspectives: The HP-PRRSV remains to be a major threat to swine production. The current modified live vaccines do not provide complete cross-protection against heterologous PRRSV strains. This presents a huge challenge to protect pigs from infections by increasingly divergent HP-PRRSV populations in the face of rapid viral evolution. This somber reality strikes an urgent need to know more about HP-PRRSV biology and its interaction with the host with the aim to make an ideal vaccine that can provide broad protection. Here we summarize some important questions to be addressed in the future. (i) What are the immunological targets for T cell-mediated responses? What constitutes a protective T cell response? What role do TREG cells play in regulating immune responses? (ii) Why do the neutralizing antibodies lack cross-neutralization ability? What have we missed from this? What receptors does HP-PRRSV use to gain access to epithelial cells *in vivo*? We need to know more about entry mechanisms of HP-PRRSV from both virus and host sides. (iii) What are the molecular mechanisms of HP-PRRSV RNA polymerase to promote replication and viral virulence? How does it promote genetic diversity and recombination in the field? (iv) How does HP-PRRSV differentially induce aberrant inflammatory responses? (v) What are the mechanisms of HP-PRRSV-induced apoptotic cell death? How do the infections of thymus and the related T cell apoptosis affect the nature and quality of newly developed T cells? (vi) How do HP-PRRSV infections predispose pigs to secondary bacterial infections? How do bacterial and other viral infections potentiate HP-PRRSV-induced pathogenesis? (vii) What role do $CD4^+$ TH17 cells play in fighting bacterial infections during HP-PRRSV infection? (viii) What are the mechanisms of reproductive failure owing to HP-PRRSV infections?

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The prevalence, prevention and control strategies of swine bacterial diseases in China

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1. The prevalence of the main bacterial diseases that mostly affected China pig industry

1.1 Singular and dual bacterial infection

Bacterial infection is a commonly observed factor leading to economical losses in pig farms located in different areas of China. Through bacterial isolation using BSA media supplementary with bovine serum and in the aerobic condition, in 2016 and 2017, in the samples collected from sick pigs of the intensive pig farms in central parts of China. Totally, in 2016 and 2017, 8226 and 9681 samples were sent for laboratory diagnosis, from which 3453 and 4593 strains were isolates. Among these isolates, the numbers of *Streptococcus suis*, *Haemophillus parasuis*, *Pasteurella, Actinobacillus pleuropneumoniae*, swine erysipelas were 704, 553, 120, 24, 21 and 1666, 696, 382, 97, 33, respectively. *Actinobacillus pleuropneumoniae* and swine erysipelas were responsible for the most acute death cases of the sows and the finishing pigs.

Table 1 The serotypes of *Streptococcus suis* strains

Year	No. of strains	Percentage of serotypes (%)									% of Non-Typable strains
		1	2	3	4	5	6	7	8	9	
2016	136	6.62	29.41	8.09	0	3.68	0	13.97	0	8.82	29.41
2017	234	5.1	28.9	8.09	1.7	2.55	0	13.19	3.4	16.17	17.87%

Table 2 The serotypes of *Haemophillus parasuis* strains

Year	No. of strains	Percentage of each serotypes (%)												
		1	2	4	5	6	7	9	10	12	13	14	15	Non-typable
2016	179	1.68	0	20.11	25.7	0	0	0	2.79	7.82	14.53	7.26	0	20.11
2017	259	4.25	2.7	22.39	28.57	0.39	0.77	0.77	1.16	3.86	13.51	3.47	1.16	16.99

From these data, it is still interesting that a large portion of the isolates are non-typing serotypes, indicating the possible recombinant strains with unknown pathogenecity.

Besides our laboratory, other institutes are also paying attention to the epidemiology of the bacterial diseases. The prevalence of different bacteria has also been reported by other research groups. The HPS positive rate of sera collected from non-immunized nursery pigs in Sichuan in 2014 was 64.10% by Tan et al. (2015). The investigation of bacterial infection in the 53 pigs with respiratory problem from 41 intensive pig farms at Sichuan province revealed the prevalence of the singular bacterium were 54.72% (29/53), 47.17% (25/53), 20.75% (11/53, 16.98% (9/53), 13.21% (7/53), 9.43% (5/53) for *Streptococcus suis(SS), Pasterella multicida(Pm), Hemophillus parasuis(HPS), Actinobacillus pleuropneumoniae(APP), Listeria monocytogenes, Pseudomonas aeruginosa, Bordetella bronchiseptica(Bb)*, respectively (Song Yin, et al., 2015) while the dual infection patterns in the same samples are 32.07% (17/53), 30.19% (16/53), 22.64% (12/53), 13.21% (7/53), 11.32% (6/53), 9.43% (5/53) for HPS+SS, SS+Pm, Pm+HPS, APP+SS, SS+ *Listeria monocytogenes*, SS+ *Pseudomonas aeruginosa.* In a retrospective study for bacterial isolation from 167 sick or dead pigs collected from 69 intensive pig farms in Guizhou province during 2008 to 2014, 75 strains (44.91%) were isolated from which 35 *E.coli* strains (46.67%) and 19 *Staphylococcus* strains were isolated, indicating these two bacteria were most common in these samples (Ma Guangqiang et al., 2015).

The results are helpful for veterinarians to decide which inactivated vaccine is more powerful in prevention of the disease caused by multiple serotypes and also useful for the researchers to start the development of the new generation of vaccine using the prevalent strains.

1.2 Dual or multiple infections between bacteria and viruses

The presence of immune-suppression caused by PRRSV or PCV2 enhanced the secondary infection by bacteria. In an analysis of 112 PPRSV-infected pigs in Sichuan province between 2013 to 2016, isolation rates of *Staphylococcus* strains and Pm, *E.coli*, APP and *Streptococcus suis* were 70%, 50%, 40%, 40% and 30%, respectively (Li Youyou et al., 2017). The case reports of co-infection by HP-PRRSV-PCV2-*Streptococcus suis* (Sun Guangye et al., 2017), PRRSV-*Streptococcus suis*-HPS (Yu Jifeng et al., 2018), Pseudorabies virus-HPS (Zhong Yi, 2017), PCV2-HPS (Fei Yuchao et al., 2017) were presented.

Through bacterial isolation and 16sr-RNA PCR, We investigated the bacterial infection patterns in the pigs that were infected with wild type of pseudorabies virus or not. The isolates are further serotyped. The findings are interesting as the low or middle virulent serotypes of HPS, *Streptococcus suis* and APP are secondary pathogens in PRV-infected individual pigs while the high virulent serotypes strains are infected without significant different rates in both PRV gE-antibody positive and gE-negative pigs. Based on these findings, the traditional concept should be modified that the low bacteria are the secondary infection after viral infection while the virulent serotypes of bacteria are not as they can directly infected pigs and may lead to the highly morbidity in the herds.

2. The technologies for the diseases diagnosis

The diagnoses of the bacterial-related disease are based on the pathogen-detection, epidemiological data, clinical signs and pathological examination. In the diagnostic laboratory, the multiplex PCR to serotype different isolates or simultaneously detect multiple bacteria in one detection reaction. We have previously developed a multiplex PCR according to the sequences coding ApxI, Outer membrane protein, Transferrin binding protein (tbp) for serotyping APP. Tang et al developed a PCR to differentiate toxic and non-toxic *Pasterella* targeting the gene coding skin necrosis toxin.

3. The antibiotic resistance of the bacteria and antibiotic substitutes

3.1 The antibiotic resistance is becoming more common and more severer

As we know, antibiotics are the most effective and direct weapons against the bacterial infectious disease. However, the outbreak of antibiotic resistance is bringing a great threat and economic losses, and becoming more and more common and severer in the livestock farming. There has been reported that the antimicrobial resistance of *Pasteurella multocida* (*PM*) has been arising across all parts of our country (China), but existing regional difference. For example, the resistance rates of *PM* to Cefalexin are 95.2% in the central region of Jiangsu, but 16% and 71% in the Sinkiang and Giamgxi, respectively. Moreover, the resistance percentage of *PM* against amikacin, streptomycin, spectinomycin, penicillin-G, gentamicin sulfate and vancomycin were more than 70% and considered to be non-effective against animal *pasteurellosis*.

The resistance of *Streptococcus suis* (*S. suis*) to antimicrobials commonly used in swine including lincosamides, macrolides, sulphonamides, and tetracycline, *has* been documented worldwide, with resistance in up to 85% of strains. Among of these examined antimicrobials, the resistance of *S. suis* to penicillin, ampicillin, and ceftiofur has been demonstrated to be (0–27%), (0.6–23%) and (0–23%), respectively. The high-level resistance rate of *Haemophilus parasuis* (HPS) to ampicillin, tiamulin, enrofloxacin, and imipenem were detected as 50%, 50%, 46.7% and 100%, respectively in China.

During 2008–2015, a total of 15,130 *E.coli* were isolated and the susceptibilities of these isolates to 9 classes of antimicrobial agents (florfenicol, sulfisoxazole, enrofloxacin, colistin, gentamicin, tetracycline, ampicillin, amoxicillin/clavulanic acid, ceftiofur) were determined. The findings of this investigation reveal that (1) multi-drug resistance was highly prevalent in *E. coli*; (2) these *E.coli* isolates showed high resistant rate (>80.0%) to several old drugs, including ampicillin, tetracycline and sulfisoxazole; (3) increasing resistance to colistin (25.6%), florfenicol (46.0%) and ceftiofur (15.9%). All these data highlight the rising problem of antimicrobial resistance. It is urgent to improve the management

and proper use of antimicrobials.

3.2 Mechanism of the resistance and the solutions to solve the problem

Resistance mechanisms vary among different bacteria. (1). Some are directed at the antibiotic itself: enzymes such as β-lactamases destroy penicillins and cephalosporins, and modifying enzymes, inactivate chloramphenicol and aminoglycosides such as streptomycin and gentamicin. (2). Others target how the drug is transported. For example, an active efflux of drug mediates resistance to the tetracyclines, chloramphenicol and the fluoroquinolones. (3). The third type of mechanism (not shown) alters the intracellular target of the drug—for example, the ribosome, metabolic enzymes or proteins involved in DNA replication or cell wall synthesis—making the drug unable to inhibit a vital function in the microbial cell.

To solve this problem, many strategies should be used. (1)The rational uses of antibiotics are the key approaches to fighting against the antimicrobial resistance. (2) In addition, it is necessary to adjust the dosing regiments for clinical treatment while reducing the occurrence of resistance to antimicrobial drugs. (3) Furthermore, it is also essential to make efforts for new drug development-either those that block or circumvent resistance mechanisms or those that attack new targets. (4) The availability of rapid diagnostics to distinguish a viral from a bacterial infection would also greatly decrease the abuse of antibiotics.

3.3 The screening and application of antibiotic substitutes are becoming popular

To reduce the residue of antibiotics in the pork and to further improve the food safety, some antibiotics are gradually prohibited in the pig production. However, for the purpose of prevention and control of the pig diseases, the antibiotic substitutes are the hotspot of the novel medicines.

Probiotics consisted of various normal flora, such as *Bacillus bifidus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Lacticacid enterococci*, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Candida utilis* and so on. (Sha Wenfeng, et al., 2015) are developed for improving the digestive system function in the sows and prevention of bacterial diarrhea. Additionally, probiotics have the advantages such as no toxicity, no residue, and no drug resistance; therefore it is widely applied in the pig industry as a green feed additive instead of antibiotics. Healthy piglets that took SRONG-PIGLET (this is a brand name of commercial product) twice a day, 5 ml once, for 2 days, the incidence of piglet white dysentery can decrease to be the lowest (3.06%); the diseased piglets that took SRONG-PIGLET three times a day, 5 ml once, for 5 days continuously, the recovery rate can achieve 95.00% (Peng XP, et al., 2013). Moreover, the recovery rate of piglet refractory diarrhea can achieve 90% after using humic acid probiotics for 6 days, while it is only 26% after using traditional medicines (Wang DW, et al., 2013).

Compared to the antibiotics, the Chinese traditional medicine including houttuynia cordata, poria cocos, hawthorn and so on could reduce the diarrhea rate (42%-69%) reported by Mao (Helai Mao, et al., 2016). The Chinese traditional medicine has the advantages such as low toxicity, residue, resistance and can promote the piglet growth effect is widely applied in the pig industry.

4. The novel vaccines for prophylaxis of the bacterial diseases

The traditional vaccines for immunological prevention of these bacteria diseases are live attenuated vaccine for swine erysipelas, swine *Pasterella* and *Streptococcus suis* (*Streptococcus* equines C-group based vaccine). Subsequently, based on the epidemiological investigation, the prevalent-serotype strains-based inactivated vaccine are developed for porcine contagious pleuropnemoniae (serotype 1/3/7; serotype 1/2/7), HPS (Serotype 1/6; Serotype 4/5) and *Streptococcus suis* (serotype 2, serotype 2/7/9) and swine rhinitis. The subunit vaccine that consisted of ApxI, II, III and Out membrane protein, is commercial available to fight against APP despite of its expensiveness.

Recently, bivalent vaccines against both HPS and swine *Streptococcosis* using two isolates or subunit vaccine are at the stage of clinical trials and is believed to be approval soon. Also, the vaccines against HPS and PCVAD are being clinically evaluated. The naturally or genetically-attenuated vaccines against *Hemophillus parasuis* is evaluated in the laboratory condition aiming to provide serotype-cross protection. The ApxIC/ApxIV double inactivated vaccine that developed by Dr. Bei Weicheng has been developed and can also elicit strong protection against different serotypes infection. More

importantly, the ApxIV-deleted live vaccine may pave the way for differentiation of vaccinated and naturally infected pigs. The principle for this differentiation test is the lack of ApxIV during *in vitro* culture of APP due to the non-synthesis of this protein while its production can be induced in *in vivo* infection of the pigs, thus leading to the presence of specific antibody against this ApxIV toxin. This antibody can be detected with the commercial kit.

5. The eradication of porcine contagious pleuropneumoniae is theoretically and technically possible.

Theoretically, like that of swine pseudorabies, the availability of gene-deleted vaccine with a combination of differentiation test kit can provide the technical support to the disease eradication campaign. However, PCP outbreak is largely associated with ventilation, pig intensity, survival and transmission of the agent in the environment. The epidemiology of APP at herd level needs further study. The concept of periodically using antibiotics in the feed to prevent the disease and therapeutically role of antibiotics in controlling PCP may block the idea of PCP eradication. But, with the compulsory of decreasing in antibody use in China and appearance of drug resistance, PCP eradication should be encouraged.

6. Conclusions

With the rapid development of intensive pig production, bacteria are the common pathogens responsible for high mortality and mobility of the pigs. The PRRSV and PCV2 facilitate the secondary bacterial infection while the highly virulent bacterial infection can be observed independent of viral infection. The application of serotype-specific vaccines, probiotics and improved environment conditions in light of animal welfare are more popular measurements to control the bacterial infection. Antibiotic substitutes cast a light for prevention, not therapeutic purpose, of bacterial infection. On the basis of gene-deleted vaccine and ApxIV-ELISA, porcine contagious pleuropneumoniae can be eradicated from the pig farms.

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The key viral proteins of PRRSV impairs the function of monocyte-derived dendritic cells via the release of soluble CD83

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Abstract:
PRRSV has a severe worldwide economic impact on the swine industry. Understanding the mechanisms by which PRRSV infection suppresses the immune system is essential to creating a robust and sustainable swine industry. The speaker will report that PRRSV infection manipulates porcine monocyte-derived dendritic cells (MoDCs) by interfering with their ability to produce proteins in the MHC-peptide complex. The virus also impairs the ability MoDCs to stimulate cell proliferation, due in large part to the enhanced release of soluble CD83 from PRRSV-infected MoDCs. They also found that the viral non-structural protein 1 (Nsp1) and its key amino acids in the ZF domain are responsible for up-regulating CD83 promoter activity. And viruses with mutations at these sites no longer inhibit MoDC-mediated T cell proliferation. These findings provide unique insights into the mechanism by which the adaptive immune response is suppressed during PRRSV infection.

Keywords: PRRSV, MoDCs, immune suppression

Ten years with African swine fever - lessons learned

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African swine fever (ASF) is one of the most severe viral pig diseases. No effective vaccines or treatments are available. For this reason, it has very serious socio-economic consequences. ASF is present in Russia since December 2007 when it was first introduced in the North Caucasus regions and over the past 10 years it spread throughout Eastern Europe, affecting domestic and feral pigs, reached the Baltic countries and became endemic. Epizooty is caused by the genotype II virus which causes up to 100 percent mortality in domestic and feral pigs and can be considered as a self-limiting disease. Experimental infections in domestic pigs and wild boar revealed a high virulence and moderate contagiousness of the virus (isolates).

Clinical signs of ASF caused by this virus are not specific and explicit, especially in wild boar. For this reason, laboratory diagnosis is crucial. From a technical point of view, the diagnosis of ASF does not present a problem: PCR tests are robust and have almost 100 % of correlation with the results of regional laboratories. The only question is a reasonable practicality of active surveillance in wild boar, given that the ratio of positive samples is extremely low.

Since the beginning of the epidemic, the virus (including isolates from the last outbreak in Siberia) has not changed significantly its molecular structure and properties in comparison with the very first strains isolated in Abkhazia in 2007. As it was demonstrated, some minor genome changes identified do not play a significant role in an epidemiological sense, and experimental infections cause the same clinical course and manifestations.

From the very beginning, there are two main driving forces of epizooty: the economy and human behaviour, whereas, the role of wild boar is still not completely understood. There are many examples demonstrating that the spatial pattern of the disease is characterized by “jumping” spread caused by illegal movement of pigs and pork products (in 2017 ASF has been registered in Siberia).

One of the main factors of ASFV introduction into domestic pigs herds is its low biosecurity: about 80% of ASF outbreaks have been registered in the backyard sector. The majority of them are linked to illegal trade and uncontrolled movements of infected pigs. It is known that wild boar plays a critical role in the introduction of the virus into the new territories through administrative borders. Meanwhile, the involvement of ASF affected WB populations in the introduction of the virus into the farms, especially into big industrial farms, have never been confirmed. Moreover, there are facts of illegal disposal of domestic pigs carcasses in the forest following by detections of ASFV in wild boar population. ASF can be relatively easily controlled in domestic pigs. At the same time, there is some evidence that after a quite long time (18 months) and a dramatic decrease of the wild boar subpopulations (80-80% of the initial population size) the disease may fade out. It also worth noting that the role of ticks in the epidemiology of ASF in Eastern Europe can be omitted.

Given the facts mentioned above, it can be assumed that theoretically ASF can be controlled and eradicated in without vaccine.

Under the influence of ASF the pig industry of Russia has changed in many aspects. It helped to review the legislation on preventive and control measures, it facilitated to significantly improve biosecurity of pig farms and to change the structure of the pork production (reduction of susceptible species populations (wild boar and domestic pigs in backyard sector) and finally, it dramatically increased pork production in Russia.

Nonetheless, despite many gaps in knowledge on ASFV, there is no (or low) interest to any scientific researches on ASF in Russia. There are only a few requests from the industry, which are mainly related to increasing of biosecurity measures and disinfection efficiency in terms of a shortened production ban.

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Outlook of pig farming in China and ASEAN countries during these 2 decade years, herd health management and food safety direction

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Topic

Introduction
China modern-modern pig farm today
Pig farm in ASEAN countries vs. "Ring of Chinese over sea"
Swine diseases-herd health-IM culture
China and Thailand episode

Introduction

The positioning of most modern pig farming in Asia in the past was in Taiwan, China which had used imported farm equipment from US with image of leader of Asia in pig farm know how in that period. Until FMD was happen in Taiwan, China in 1997 it was impact to era of main export pig and pork to Japan was end. Pig farming in Taiwan, China was collapsed and continue to only domestic consumption. Thailand pig farm carried on Chinese know how with on time development of country and economic grew up scale, country opened wide for pig farm technology transfer from Europe and US made Thailand came to be leader of pig farming after that. Thailand was the most attractive animal health market (poultry pig and aquaculture) in that time too and be the hub of pig farming knowledge for new modern pig farming in 7 countries around Thailand by the map (ASEAN countries) to follow.

China the biggest pig production in the world with totally changed its structural and concept of farming as the pyramid upside down. It made you need to know the new words "China modern and modern pig farm". Because of Great China, ASEAN 8/10 countries are continue growing fast of economic, tourist, value, consumption, and productivity with well balance in between 3 religions and of course pork is delight dish in various Asian cuisine.

China modern-modern pig farm today

The first significant sign of pig farming revolution appeared in 2009-2010 in eastern provinces surrounding economic Shanghai-megacity and Guangdong with fully farm layout-modern closed housing system and automatic equipment as the duplication from US-EU. Adjustment of farmer in new investment had come because of main reason, those damage of productivities between 2006-2008 from high pathogenic PRRS diseases, lack of pig to market spring up the high price high margin of finisher output. But that time 100% of new modern pig farm could not prevent their farms from the new variant of PRRS virus. The outbreak came from broken bio-security fence and wrong pig flow include did not really know enough of the existing of this virus vs imported GGP-GP which clean from Chinese's pig diseases. The other big farms in same area also expand the scale and mixed previous GP-PS to new import GGP-GP and faced to local diseases flow too.

Other side the domestic breeder and big scale leader group also progress in multiplication of PS sustained in market and more successful because of more resistant to local diseases (2011-2013). Include the modification of pig barn-pig housing to achieve ventilation and warm in winter time and flow pig in small group as all in-all out system in new farm bigger scale, these groups also doing the mass production output. With better profit, these local groups could increase the farm scale and many branches located in many provinces.

Learning of loss from HP-PRRS diseases (2006-2009) gave a good lesson of practitioner and farm owner. So the new modern farming during 2014-17 emphasized rigid bio-security, negative nucleus herd from PRRS-PCVD-CSF, perfect farm layout, less use of PRRS live vaccine, limit immunization per year, use stronger and resistant breed in importing,

high number piglets in litter. These are key factors which learnt from experience.

In fact, between 2013-2017 these 5 years Chinese producer went to shopping pig breed over EU countries and US-Canada and could help the genetic companies in US and EU recovering from bankrupt and came to be strong in financial today. Chinese producer learnt by experience and improvement to run pig business as negative from target important diseases. New farm located far away in good isolation place and could control diseases better.

The China government policy in keep the water reserve clean from pollution. And old farm area or high pig density area which surrounding the city zone were asked to withdraw and stop the activity. This policy also made 2014-15-16 pig & pork supply was not enough sudden and informal allow pig import around the border line of Vietnam-Lao-Thailand-Myanmar. It caused 4 neighborhoods supply increase sudden pig price was good all region. But when China closed import , market in 2016-17 was pig price down turn over main pork consumption in ASEAN except The Phillipines.

Next step of China government is direction of Food safety which needed to achieve for country's huge consumption of safety pork. This matter made the prediction comes true that Great China, main dish of pork consumption of the world would be key influence to ASEAN in demand and supply in many things many items include effect to pig market and animal health products in nearly future.

Pig farm in ASEAN countries vs Ring of Chinese oversea

Economic growth together with better quality of life such as Singapore Thailand Malaysia Indonesia follow with Vietnam Cambodia Lao Myanmar, also can see pig market growth too.

Thailand = Pig knowledgeable, was well known as a hub of knowhow in pig farming. Strength for years those are; best farm management in efficiency and cleanliness in this region. Leader in PSY and pay attention in people skill. Evaporative cooling system housing for all age of pig, AI boar, pregnant-lactating sow, nursery, 2-sites production (wean to finish) and fattening, early weaning as 22-23 days average (range 20-24). 30-40% of country use 2-sites production (means no nursery housing). AI 100% Duroc boar (pure or D+PT) is most popular, AI boar in isolate evaporative cooling house system and control diseases well. High density pig area, some use P0-P1 special unit. Biogas system and generate electricity and produce LPG. In the past: at farm's gate to buyer truck, but today: farm to out let, It is moving fast.

Vietnam = Pork favorite and market growth, its consumption is increasing every year. It passed a long period (of tough, low profit, slow down economy) to be upgrade pig farming modern today. History of pig farming development: In 1st phase and 2nd phase, many foreign countries investment had loss profit and back home, the left stand still today be firm in new economic growth of Vietnam (>7% these couple of years). Only one country in ASEAN which has high magnetic draw investment from all regions of the world came here, it's hottest of pig farm investment during 2014-2016, genetic companies and farm technology are input here. Top two ranking are CP company from Thailand and Japfa Comfeed from Indonesia. The strength is labor cost and labor skill so far so good, pig knowledge is gradual increase. The scale of business and framework of pig farming might be quick change from now. Of course after pig price down, took over or hand the farm over to the winner it would happen. Although pig price down turned in 2016-17 because of China closed border line. Another reason is fulminate growth of big scale farm while slaughter house and outlet still not move forward for Vietnam. Nevertheless, this is not really crisis but a kind of "reset" and "reform" the basement of pig farming system to be professional business similar to other segment of business in Vietnam which fast growth.

Cambodia = Major pig farm area are surrounding capital Phanom Penh, biggest farm size belong to CP PNP company investor from Thailand, second ranking is M-Pig local farm company down south of PNP. Economic growth induce demand of consumption, but CBD locate in between big pig volume countries as Thailand at west Vietnam at east, both send pig in if too much different gap of pig price. New pig farm in CBD always closed if not much money to maintain at low price period. Small holder is easy come and easy go.

People Democratic Republic of Lao = Population not many, peaceful country and economic moving up, many investment from outside country which drive the PDR Lao to be sudden change these 2 years, major investor are China and Thailand. Pig farming number one is CP company, second is Vientian local farmer. Thai pig farmer and farm company are setting pig farms there. Chinese people are so many there, hope that number of pig farm should increase

soon.

Myanmar = new open country and start up many thing also pig farming, 1,000-2,000-4,000 sow scale were set up in north, at new capital city, old farm area in around old capital and south west. Farming system implement in foreigner investment, some include feed mill. It look like the country in preparation stage for new consumption.

Malaysia = walking in the pig farm there seem to be in Taiwan, China, because Chinese oversea there still use Mandarin which pronunciation similar Taiwan, China. Farm structure, pig barn prefer open house because climate sudden change like the oceanic climate, ventilation and fan are most important compare to evaporative cooling system. Chinese character is everywhere, culture of people, restaurant, sea food, make you feel as in Taiwan, China. Pig farm locate mainly in Penang Epoh and north of peninsula, east MLS are in Sabah and Kuching. Low density area no PRRS, high density pig area some use PRRS vaccine. The government allow import pig and semen from Denmark only. 2,000-4,000 farm scale increase these couple of years**.**

Indonesia = raising pig area also same as MLS where are Chinese oversea or Chinese blood community there are pig farms. In Indonesia mainly pig farming there are in Maidan, Jarkata, Solo, Bali, Ambon etc. where is more Christian and Buddhist area. Pig farm know how and step of animal health market follow step as Thailand. Because of CP's basic there.

The Philippines = Isolate surrounding by the sea, limit diseases, pig farming area not so much increase number, pig farm association are strong, farmer are relax and take it easy. It had changed and growth but not so fast speed. There are many modern farms and famous top ranking of ASEAN too.

Ring of Chinese oversea: As we know the Pacific ocean's rim surround in circle between continental called "ring of fire". Beneath it, there is a small ring of peaceful places belong to many countries, enrich with natural resort this ring locate from Hainan island (a province of China main land) pass down south to Hanoi, Hochimin city, anti clock to Phanom Penh, Bangkok Metropolis, Hat Yai, Penang, Epoh, Kuala lumper, Singapore, pass Jarkatar mega city upward to eastern Malaysia Salawak, Sabah, Kuching-Kota Kenabaru, Brunei to Manila mega city and up north back to Chinese Taipei. It is an interesting ring more than 300 years ago. Chinese migration from Guangdong and Fujian mainly. Where is Chinese over sea, there is Chinese business, merchant, restaurant, pork and pig farm. IPVS this times take place in right place.

In conclusion China and ASEAN have deep in long time connection as ring of Chinese oversea, ring of pig farming.

Swine diseases-herd health-IM culture

Swine diseases in this region: category I, II, III, IV, V

Category I. Emerging diseases and re-emerging diseases.

Swine infectious diseases in the past are CSF., AD., FMD, and emerging diseases are PRRS., PCVD., PED. The situation in each country are different not in the same at all because of scale, density, culture of using AH product, the way of thinking, limitation of investment, knowledge-know how-back ground, climate, raw material etc. Good example in swine diseases link in region, is a disease which could spread over many countries in same region via transportation route and across the sea that diseases is HP-PRRS. Which 2009 was spread from China to southern Vietnam and Cambodia. Within one year it develop to be 2010 isolate in northern Vietnam and spread to People democratic republic of Lao and in short period by piglet-finisher commercial interaction between the Thai-Lao border line, this disease passed through north-east part of Thailand and spread to northern part then at the end of year 2010 it spread to Myanmar. All samples were sequencing and could trace back to 2009 origin source.

Similar work out also told us that PED had spreading across the sea as outbreak in Korea(2005-06)- Philippines-Thailand(2007-08)- Vietnam-China(2010)-US.(2014)-Japan (2016). FMD new strain which great different from vaccine strain produced by each country, also outbreak found wide spread in this region (2012-2017) appeared as spot in cattle-pig farming, at port, import quarantine place etc. Popular beef consumption hit in Korean restaurant- Japanese restaurant are increase beef consumption over ASEAN-China. Cattle migration and movement pass each country across the sea made FMD wide spread over region.

Emerging diseases and re-emerging diseases, its picture of spreading and jumping (as bird migration) told us that Economic growth increase consumption and business market new type, new born town and mega city,

business-industrial MOU between countries, quick transportation-logistic, your pig diseases come to be my pig diseases also mine to be yours. It's time for every country to open the door to talk (not build the wall) find possibility centralize vaccine production and action together (just think why US president ask to stock this vaccine for country emergency), good solution of each country could share and implement.

Category II. Common diseases in pig farm (which can reduce by sanitation, divide group, all in-all out, Oxygen-ventilation)- vertical sow&piglet- via AI – (pathogen use farm to be their habitat).

MMA after farrow, colibacillosis, clostridium, salmonella, PM., SS2. PPV., BB.(AR), MH.

Category III. Secondary infection-PRRS (PCVD) induced complex diseases- opportunist by MCT predisposing those are;

Secondary infection-PRRS (PCVD) induced complex diseases: PM., SS2., MHR., HP., SD., Li., erysipelas, AR. (include CSF fare up in endemic farm)

Opportunist by MCT predisposing: Actinommycess pyogenase, H. parasuis, Meloidosis

Category IV. Diseases come by seasonal-climate-carrier-virgin first times approach.

There are Swine Influenza, JE., Pox, APP, Leptospirosis, Toxoplasmosis (Brucellosis) .

Category V. Diseases contaminated in biological products- live vaccine-autogenous vaccine.

Contaminated in cell culture, media: BVD, MH, PCV-1, Delta virus B, Seneka A virus?

Live vaccine strain turned some virulent some times: PRRS, CSF

Insufficient killed**:** Erysipelas, Leptospirosis and some local viral KV

Herd health were handle by experience Vet, consultant, university professor, expert in Asia, expert from EU, US, Australia. Herd health control program popular use in government concern standard farm and company farm and private scale more than 2,000 sows up. Content of herd health in the past are concern in diagnosis, find the real causative agent, find the loop outbreak, treatment immunization, prevention, bio-security, standard farm.

Today more content are: free from target diseases herd, mycotoxin more concern, genetic susceptible-resistant animal aim select to multiply, reduce use of antibiotics, alternative choice to replace antibiotics, many choice to build up health, mucosal immunity, use more pro-biotic, withdraw medicine more month, AMR from farm to human.

IM culture, IM is intramuscular this means injection. It is treatment and immunize pig. A small holder there is no antibiotic in the feed nor growth promoter in maize feed. When a pig get sick there are 2 choices treatment by inject medicine and sold out or kill to be food. A pig farm more pig number as a herd with different group of pigs in each barn, some housing are crowded, use growth promoter and use medicated feed. Any day if found sick pig it would be inject with antibiotic which different kind from antibiotic in feed.

A commercial big pig farm or company it was high investment its target of productivity was set high, in every day they have injections both medicine and vaccine, more found sick pig more use antibiotic and increase number of vaccine shot, pig loss or died mean loss profit.

After be back from business seminar there are more items more kind of vaccines must be insert in the routine program (plus more), they do mass vaccination 2-3 items in month and in week program of sow herd. In nursery pig when no gap to insert any vaccine any more, they was advised to move some vaccine program to do faster through all sucking period which every 5-7 days one disease' s vaccine inject to suckling piglet. A lot of needle were used every day in big farm scale, pig must be inject, injection are main task of each day so why we call "pig farm a land of intra-muscular or Land of IM" come to be culture in pig farm in this region. Fear to loss profit, afraid it would be big outbreak happen or fast spread. So this culture probably came from Chinese blood, diligent, hurry up, don't want to loss, not damage, save pig back and fear. If found 3% of problem because of "fear" make to hurry up do the same thing of 3% to all 97%. This example lead to excess use of vaccine and drug too much. Please change the way of thinking, change IM to be MI mucosal immunity!

China and Thailand episode

China first decade year 1997-2007: learn to use premix, to understand feed and feeding, learn each swine disease, what is PRRS, PCVD? how to diagnose, how to treat how to use a drug, how to use vaccine what is vaccination program. From natural mating to do AI without fear (do not afraid that not pregnant nor low litter). " save more pig is profit, let's do

treatment so IM" pig price very low. Then 2006 HP-PRRS damaged big loss lack of supply and pig price up high sky rocket.

China second decade year 2008-2018: Trend to be genetic improvement in China pig society that's why China country is a biggest customer of Genetic seeker.

a) Pork consumption: need fast growth pig breed and more lean meat to market.

b) Country sky rocket economy-biz growth in hand-new richer, new pig farm increase scale as pyramid upside down.

c) Mass production style: learnt to have huge profit in hand, then step to want to occupy the market share, changed themselves to be more integrating framework.

d) Serve growth of mega-cities, city life style and step to control consumption and outlet (fast food- brand name- Korean food-Japanese food- Franchise loop).

Pig Biz in China thought the way to import GGP-GP is to use "quick solution" to reach the goal and fast expansion the scale. It's the answer of occupation the market share and fast move to high ranking. So China today is a big pool of almost famous world pig genetic companies input together, "China is world pig pool of genetic". In the same time is a stage of show time which genetic will pass examination or not!! Domestic pig diseases and imported pathogen also pool together.

In the past China use high number of sow to produce piglet, today those number reduce with trend to use higher PSY genetic to compensate. Get rid of PRRS, CSF, AD, AR, FMD from GGP-GP level is 1st priority. PED need to use alternative choice of "preventive medicine" to use pro-biotic induce mucosal immunity (IgA for PED, the real protection) to piglet and use Colostrum management to protect whole litter from PED infection during suckling period. Modern pig farm in China's aim to keep high productivity & safe high litter piglet, weaned till market with limit low loss on the way. Modern pig farm's philosophy is a kind of business at junction between Agriculture vs Industry. Means while, people skill development and continue improve staff's knowledge and efficiency (people-ware) still be important key point.

Thailand pig farming after post-modern period

Pig farm equipment "Modern pig farm" did not mean to copy housing equipment from EU or US 100% nor use expensive tools to replace worker. Hot country raise pig in evaporative cooling system today the result of productivity and performance are closely to result in cold country and genetic had long time adapted already.

From 2000-today, emphasized sow comfort, feed intake, litter size, PSY, fattening pig comfort, ADG-FE, and cost of production.

2014-today, mucosal immunity and colostrum management is key helper to increase PSY and reduce loss on the way. Company farm changed to more integrate business, cover slaughter house, meat processing and outlet.

Trend from 2017-up, bio-security is major concern, new direction was clearly to reduce diseases inside and reduce use of antibiotics. Coming of food safety, ban use of colistin, AMR project co-operation to reduce resistant in human.

PRRSV control – the Danish way

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Keywords: PRRSV, disease control, vaccination

PRRSV has since its first appearance in the beginning of the 90 ties been one of the major health challenges in the Danish pig production. According to figures from the Danish SPF society, approximately 35 % of the Danish pig herds are positive for PRRSV, and the prevalence of positive herds are declining. Both PRRSV-1 and PRRSV-2 is prevalent in Danish herds and some herds are infected by both species.

There is a variety of PRRSV programs in place for the control of PRRSV in different herds, but the preferred strategy for most herds is to establish a stabile sow herd where the sow are immunized/exposed prior to introduction into the sow herd and the piglets are PRRS virus free at weaning. In contrast, most herds play less attention to virus circulating in the nursery and among growers/fatteners. Sow/mass vaccination is used in many herds, whereas piglet vaccination is less commonly used.

The presentation will give a detailed presentation of the Danish PRRSV control system with focus on which control measures that works and on procedures where there is room for improvement. Results from laboratory experiments, *in vivo* challenge experiments and field trials will be presented. The focus of these studies have been on aspects related to external and internal biosecurity (purchase of semen, gilt acclimatization strategies, AI/AO, sectioning, pig flow etc.), pros and cons on the use of vaccination, impact of genetic and antigenic diversity, virulence of circulating strains, infection dynamics and diagnostic procedures.

Finally, perspectives on the future strategy for the control of PRRSV will be discussed seen in the light of the growing public concern on the use of antibiotic in the pig production. Is it realistic to eradicate PRRSV on the national/regional level?

Mycoplasma hyopneumoniae infections: update on pathogenesis, vaccination and control

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Introduction

Mycoplasma hyopneumoniae (*M. hyopneumoniae*) is the primary pathogen of enzootic pneumonia (EP), 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 derived from secondary infections (Holst et al., 2015). The present paper provides an update on current knowledge on *M. hyopneumoniae* infections, with emphasis on pathogenesis, vaccination and control of the disease.

Pathogenesis

M. hyopneumoniae is a host specific pathogen that only infects pigs. Upon inhalation, *M. hyopneumoniae* organisms penetrate through the mucous layer and adhere to the ciliated epithelial cells of the trachea, bronchi and bronchioles.

The process of adherence to the cilia is complex. It is initiated by aspecific hydrophobic interactions, followed by specific interactions between adhesins of the mycoplasma cells and receptors on the membrane of the cilia. The primary adhesin of *M. hyopneumoniae* is the P97 and its paralogues (Hsu et al. 1998). The other family of adhesins, related with P97, is formed by P102 and its paralogues (Adams et al. 2005). P159 is an adhesin unrelated to the other two (Burnett et al. 2006). Most of the proteins from the P97/P102 paralog families and P159 are post-translationally processed and cleaved, a system observed with many other surface-associated proteins (Seymour et al. 2010). P146 (LppS) is an adhesion lipoprotein which is also proteolytically processed (Bogema et al. 2012).

Tacchi et al. (2016) identified 35 proteins that are endoproteolytically cleaved in *M hyopneumoniae*. These include adhesins but also lipoproteins and even multifunctional cytosolic proteins “moonlighting” at the cell surface. The cleaved fragments of the P97/P102 paralog families and P159 remain on the cell surface and function as receptors of glycosaminoglycans, plasminogen and fibronectin, thereby influencing interaction of *M. hyopneumoniae* with its host (Bogema et al. 2012, Simionatto et al. 2013). This massive processing and cleavage leads to a very dynamic surface topography of *M. hyopneumoniae* that could be involved in host evasion and modulation of the immune response.

Within hours after the adherence of *M. hyopneumoniae* to the cilia, cilia start to tangle, clump and split. Later, cilia loss is observed before disruption of the epithelial cells. This results in a reduced clearing capacity and makes the animal susceptible to secondary bacterial and viral infections (Park et al. 2016a).

The mechanisms by which mucociliary function is destroyed, are poorly understood. *M. hyopneumoniae* causes an increase of cytosolic Ca^{2+} concentrations both in neutrophils and in respiratory epithial cells (DeBey et al. 1993). This increase can influence other pathways leading to the damage seen to the cilia. Aggressins implicated in this process include nucleases, proteases and glycerol phosphate oxidase. Cell-surface lipoproteins, also called lipid associated membrane proteins (LAMP), have been found to be implicated in apoptosis, and can activate production of nitric oxide (NO) and reactive oxygen species (ROS) in the host cell. Data generated by sequencing and comparative analysis of three strains of *M. hyopneumoniae* indicated the presence of an integrative conjugal element in two strains that were pathogenic. This suggests that the element is a mobile DNA element that could be involved in genetic recombination events and pathogenicity (Pinto et al. 2007). In addition, proteins recognized by the immune system during infection by *M. hyopneumoniae* have been described (Simionatto et al. 2012; Marchioro et al. 2014).

Mycoplasma hyopneumoniae organisms stimulate alveolar macrophages and lymphocytes to produce pro-inflammatory cytokines that play a role in lung lesion development and lymphoid hyperplasia (Meyns et al. 2007), suggesting the

involvement of the immune response in the development of lesions. On the other hand, there is also evidence for the induction of anti-inflammatory cytokines and the suppression of inflammatory cells (Asai et al. 1996).

Experimental studies have demonstrated that *M. hyopneumoniae* can persist in lungs for a very long period, at least 214 days, and that infected pigs also remain contagious during at least this period (Pieters et al. 2009). To this end, the organism needs to adapt to changing and possibly hostile environments of the host.

Although *M. hyopneumoniae* is primarily a respiratory pathogen, it has also been isolated from internal organs, suggesting that spread may occur via the lymphatic or blood circulation (Marois et al. 2007). The agent was re-isolated from the liver, spleen and kidneys of experimentally infected and contact pigs (Marois et al. 2007; Marchioro et al. 2013) and *M. hyopneumoniae* DNA was detected in these same tissues (Woolley et al. 2012). However, the spread within the body is transient and no association has been shown between the presence of *M. hyopneumoniae* in these organs and lesions.

Development of clinical pneumonia is dependent on the number of organisms in the respiratory tract, the virulence of the infecting strain(s) of *M. hyopneumoniae*, and the involvement of other respiratory pathogens. The number of organisms colonizing a pig is likely dependent on cumulated infectious doses, capacity of the *M. hyopneumoniae* strain(s) to multiply in the lungs, and time. Strains of *M. hyopneumoniae* also differ in virulence with high-virulence strains inducing more severe pneumonia in a larger proportion of pigs (Vicca et al. 2003; Meyns et al. 2007; Woolley et al. 2012). Highly virulent strains may have a higher capacity to multiply in the lungs and induce a more severe inflammatory process (Meyns et al. 2007). It remains to be confirmed whether highly virulent strains have a higher capacity for host evasion, expression of virulence associated genes, modulation of the immune response, and production of toxic metabolites like H_2O_2 compared to low virulent strains.

Interaction with other pathogens

Different studies have shown that combined infections with *M. hyopneumoniae* and other pathogens increased the severity of disease. In the past, studies mainly focussed on the interaction with parasitic (*Ascaris suum*) and bacterial infections (*Pasteurella multocida, Actinobacillus pleuropneumoniae*), whereas more recently, the emphasis has been placed on interactions with viral infections. Under experimental conditions *M. hyopneumoniae* significantly prolonged and increased the severity of PRRSV-induced pneumonia (Thacker et al. 1999). Dual infection studies with *M. hyopneumoniae* and swine influenza virus could not show such potentiating effects of both pathogens. The effect was less pronounced, only transitory and dependent on the subtype of swine influenza virus (Thacker et al. 2001; Yazawa et al. 2004; Deblanc et al. 2012). The interaction with porcine circovirus type 2 (PCV2) is also not consistent. Opriessnig et al. (2004) showed that *M. hyopneumoniae* infection potentiated the severity of porcine circovirus type 2 (PCV2)-associated disease, whereas Sibila et al. (2012) could not demonstrate such interaction between *M. hyopneumoniae* and PCV2 infection. Combined infections under standardised experimental conditions are valuable, but they only partially reflect the complexity of PRDC as occurring under field conditions. Many different pathogens may be involved under field circumstances, and environmental conditions may largely influence the disease outcome. Finally, Pósa et al. (2013) showed that pigs receiving feed contaminated with Fumonisin B elicited more severe lung lesions upon *M. hyopneumoniae* challenge infection compared to pigs fed with non-contaminated feed. Michiels et al. (2016) however did not observe more severe disease and lesions upon experimental *M. hyopneumoniae* infection in pigs that received feed contaminated with the mycotoxin deoxynivalenol (DON) at a moderately high concentration of 1,800 µg/kg than pigs fed with non-contaminated feed.

Control

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 (for overview, see Del Pozo Sacristan 2014). Also factors different from housing and management conditions, such as strain differences, may determine the infection pattern, clinical outcome and the severity of lung lesions (Vicca et al. 2002; Michiels et al. 2017).

The introduction of *M. hyopneumoniae* naïve gilts into endemically infected farms represents a significant challenge for the incoming gilts (Nathues et al. 2013) 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 (~50 days of age) to *M. hyopneumoniae*, aiming gilts to recover and become immune prior to entering the sow farm, and to no longer shedding the bacterium (Pieters and Fano 2016).

Preliminary studies have shown that selection for disease resistance may be helpful in the control of *M. hyopneumoniae* infections (Borjigin et al. 2016a), although positive effects are not consistent (Borjigin et al. 2016b).

Strategic medication in chronically infected herds has also been used successfully. Such medication programs should be discouraged because of the increased risk of antimicrobial resistance development and the risk for antimicrobial residues in the carcasses at slaughter. *M. hyopneumoniae* is intrinsically resistant to beta-lactam antibiotics, sulfonamides, antibacterial diaminopyrimidines (such as trimethoprim), polymyxins and 14-membered macrolides. Acquired resistance of *M. hyopneumoniae* has been documented for tetracyclines, 16-membered ring macrolides (tylosin, tilmicosin), lincosamides and fluoroquinolones (Vicca et al. 2007; Thongkamkoon et al. 2013; Tavío et al. 2014). Results of treating field cases of EP may be disappointing because the disease signs and the shedding of micro-organisms may reappear after cessation of the therapy. In some cases, therapy failure may be due to intrinsic or acquired resistance in the bacterial species playing a role in secondary infections.

Vaccination

Vaccination is widely applied worldwide to control *M. hyopneumoniae* infections. Commercial vaccines mostly consist of inactivated, adjuvanted whole-cell preparations that are administered intramuscularly (Maes et al. 2017). Needle-free intradermal vaccination is also possible with some vaccines (Beffort et al. 2017). Table 1 gives an overview of different commercially available *M. hyopneumoniae* bacterin vaccines. An inactivated vaccine based on soluble antigens of *M. hyopneumoniae* has also become commercially available recently (Park et al. 2016b). It is a one-shot vaccine combined with PCV2 that can be administered to piglets from three weeks of age onwards. In addition, attenuated vaccines against *M. hyopneumoniae* have been licensed in China (Feng et al. 2013) and Mexico.

Vaccination reduces clinical signs and lung lesions, improves performance, reduces the number of organisms in the respiratory tract (Meyns et al. 2006; Vranckx et al. 2012b) and decreases the infection level in a herd (Sibila et al. 2007). However, studies under experimental (Meyns et al. 2006) and field conditions (Pieters et al. 2010; Villarreal et al. 2011b) showed that vaccination conferred only a limited reduction of the transmission ratio of *M. hyopneumoniae*.

The exact mechanisms of protection are not yet fully understood, but systemic cell-mediated immune responses are considered important for protection (Marchioro et al. 2013). Vranckx et al. (2012b) reported a lower infiltration of macrophages in the lung tissue in vaccinated animals upon infection with *M. hyopneumoniae*, indicating that vaccination modulates the immune response following infection. The importance of local mucosal antibodies remains unclear. Serum *M. hyopneumoniae* specific antibody titres obtained after vaccination are not suited to evaluate protective immunity.

Different vaccination strategies have been adopted, depending on the type of herd, production system and management practices, infection pattern and preferences of the pig producer. Vaccination of young pigs, applied once or twice, is most commonly used. Single vaccination at either 7 or 21 days of age was efficacious in a pig herd suffering from clinical respiratory disease during the second half of the fattening period (Del Pozo Sacristan et al. 2014). Recent experimental (Arsenakis et al. 2016) and field studies (Arsenakis et al. 2017a) showed that vaccinating piglets three days prior to weaning conferred slightly better results than vaccination at weaning, possibly because of less interference of weaning stress. Sibila et al. (2008) and Arsenakis et al. (2017b) showed that vaccination of sows at the end of gestation, resulted in a lower number of *M. hyopneumoniae* colonized piglets at weaning. Pigs from vaccinated sows also had a lower number of *Mycoplasma*-like lung lesions at slaughter (Arsenakis et al. 2017b). Breeding gilt vaccination is recommended in endemically infected herds to avoid destabilization of the breeding stock immunity (Bargen 2004).

Many research studies focus on the development of new vaccines that may offer a better protection against *M. hyopneumoniae* infections. In one study (Villarreal et al. 2009), infection with a low virulent *M. hyopneumoniae* isolate did not protect piglets against infection with a highly virulent *M. hyopneumoniae* isolate one month later, suggesting that low virulent strains might not be suitable as such to be used as vaccines. Further research, however, is needed. Several studies have evaluated recombinant proteins of *M. hyopneumoniae* in various forms of administration and formulations (antigens, adjuvants, vectors) (Maes et al. 2017). Most of the recombinant proteins were evaluated in mice, and from those tested in pigs, only a few of them assessed efficacy upon challenge infection. Validation of the efficacy of promising experimental vaccines in pigs is needed. In order to be applicable in practice, aspects such as ease of administration and possible combination with other vaccines are important.

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Table 1: Most commonly used commercially available *M. hyopneumoniae* bacterin vaccines – Bacterin vaccines available in only one or a few countries are not included the table (Based on Maes et al., 2017)

Vaccine	Antigen / Strain	Adjuvant	Route of administration	Age of administration (days)	Boosts needed after …weeks
Hyogen (Ceva)	Ceva strain BA 2940-99	Imuvant (W/O J5 LPS)	IM	≥21	-
HYORESP (Merial)	NI [a]	Aluminium hydroxide	IM	≥5	3-4
INGELVAC MYCOFLEX (Boehringer Ingelheim)	J strain isolate B-3745	Impran (water-in-oil adjuvant emulsion)	IM	≥21	-
M+Pac (Intervet Int.) [b]	NI [a]	Mineral oil and Aluminium hydroxide	IM	≥7	3-4
MYPRAVAC SUIS (Hipra Lab)	J strain	Levamisole and carbomer	IM	≥7-10	3
PORCILIS M. HYO (Intervet)	Strain 11	dl-α-tocopherol acetate	IM	≥7	3
Porcilis PCV M. HYO (MSD-Intervet Int) [c]	J Strain	Mineral oil and Aluminium hydroxide	IM	≥21	-
Porcilis MHYO ID Once (MSD-Intervet Int.)	Strain 11	Paraffin oil and dl-α-tocoferylacetaat	ID	≥14	-
STELLAMUNE MYCOPLASMA (Eli Lilly)	NL 1042	Mineral oil and lecithin	IM	≥3	2-4
STELLAMUNE ONE (Eli Lilly)	NL 1042	Amphigen Base, and Drakeol 5 (mineral oil)	IM	≥3	-
SUVAXYN M.HYO [d] (Zoetis)	P-5722-3	Carbopol	IM	≥7	2
SUVAXYN MH-ONE [e] (Zoetis)	P-5722-3	Carbopol and squalane	IM	≥7	-
SUVAXYN M.HYO – PARASUIS [f] (Zoetis)	P-5722-3	Carbopol and squalane	IM	≥7	2

a No information available

b Vaccination scheme when one ml is used for each administration. No boost vaccination needed if a 2 ml dose is used the first time.

c Combination vaccine with Porcine Circovirus type 2

d Named Suvaxyn RespiFend MH in USA

e Same name is used in the USA, but Amphigen is used as adjuvant in the USA, and vaccine can be administered from day one of age onwards

f Combination vaccine with Haemophilus parasuis - Named Suvaxyn RespiFend MH HPS in USA

Virulence and immunity in PRRS virus infection

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Keywords: porcine reproductive and respiratory syndrome virus, virulence, innate immunity, adaptive immunity, immunopathology

Early studies on porcine reproductive and respiratory syndrome (PRRS) made evident that the disease and its causative agents – PRRS viruses (PRRSV) - had no parallel among swine diseases. It is not only the fact that two related, but different, viruses emerged in two continents almost simultaneously causing a similar disease; the disease itself and its immunopathogenesis is still far from being fully resolved. In the present talk, virulence and immunity against PRRSV will be reviewed as two sides of the same problem.

PRRSV viruses may cause from asymptomatic to lethal infections depending on a number of factors. The first factor is the strain infecting the pig. At present it is considered that PRRSV2 strains are, in general, more virulent than PRRSV1, although this is a somewhat partial picture. Some PRRSV1 isolates, for example the PRRSV1 subtype 3 strain Lena, produce a serious disease of higher virulence compared to subtype 1 isolates. In contrast, some PRRSV2 isolates may produce a very mild disease. The genetic basis for this different virulence is thought to be multigenic and the result of: a) the damage caused by the virus, b) the damage caused by the immune response of the pig and, c) the interaction of the virus with the functionality of the immune system. In other words, the damage to the host is the sum of: the direct or indirect damage to target or bystander cells caused by viral replication (necrosis/apoptosis), the damage to the tissues caused by the inflammatory response (for example, interstitial pneumonia or placentitis) and the interference with the innate immune responses (for example, inhibition of the interferon I production). With the very highly pathogenic isolates, inflammatory responses seem to be very important to explain the unusual lesions and mortality. Besides this, the participation of complicating agents, particularly in the respiratory disease, is an important factor to be considered.

The adequate sensing of the pathogen by the innate immunity and a correct antigen presentation are crucial for an efficient adaptive immunity to develop. In the first models of infection with PRRSV was already clear that the adaptive immunity against the virus did not follow the pattern of a common acute viral infection. The infected animals developed long viremias – up to several months in the born viremic piglets - and after the cease of the viremia the virus could be found in the lymphoid tissues for weeks or months. The examination of the humoral and cell-mediated immunity against PRRSV showed that although antibodies may appear soon after the onset of the infection (about 50% of the pigs are seropositive by day 7 of infection); those antibodies devoid neutralizing capacity. Actually, neutralizing antibodies rarely appear before the fourth week of infection. Cell-mediated immunity measured as lymphoproliferative responses or the development of IFN-γ secreting cells appeared relatively soon (2-4 weeks after the onset of the infection), but showed and irregular behaviour for weeks before stabilizing. To add confusion to this picture, the cross reactivity of neutralizing antibodies or of the cell-mediated immunity with heterologous strains is mostly unpredictable although some advances have been done in this area. With this picture about the adaptive immunity, the logical focus of research was to study the early events of the innate immunity.

Early studies on the interaction of PRRSV with macrophages – the main cell target of PRRSV- showed that, in contrast to other viruses, PRRSV did not induce type I interferon responses and even inhibited the responses induced by other viruses. Similarly, this phenomenon was reported in susceptible or transfected cell lines, in bone marrow-derived dendritic cells or in monocyte-derived dendritic cells. The inhibition of type I interferons *in vitro* was attributed mostly to the action

of some of the non-structural proteins of the virus.

However, these results were somewhat at odds with the detection of IFN-α in serum, tissues or fluids of infected animals. A finer examination of this phenomenon showed that when plasmacytoid dendritic cells –the professional antiviral dendritic cells that are not susceptible to PRRSV- were exposed to virus they produced IFN-α and while some highly virulent PRRSV2 isolates may inhibit partially this ability, most PRRSV1 and some PRRSV2 did not. These observations composed a more complex picture and indicated that the effects on the cytokine patterns depended on the cell type and on the strain. In fact, IL-10 and TNF-α response to PRRSV by macrophages and dendritic cells seem to be dependent on the strain. At least for TNF-α, viral non-structural proteins have been shown to be involved in this regulation.

In addition, *in vitro* models of PRRSV infection with bone marrow dendritic cells or monocyte-derived dendritic cells showed that the virus may alter the expression of molecules involved in antigen presentation (for example SLA-I, SLA-II or CD80/86) but again this seemed to be related, at least partially, to the isolate used in the experiment.

Another important question to understand PRRSV immunopathogenesis is whether regulatory T cells arise during the course of the infection. The specific literature suggests that for PRRSV2, this phenomenon is likely to may happen, although some controversial data exist. For PRRSV1 there are not such clear evidences.

Moreover, it is worth to mention that PRRSV also has an impact on the phagocytic capabilities of macrophages and neutrophils. It has been shown that the mere interaction of inactivated PRRSV with CD169 (porcine sialoadhesin) may result in an impairment of the phagocytic response of macrophages. Interestingly, it was shown that during the course of PRRSV infection, neutrophils suffered a downregulation of the expression of mRNA of FcγRIIIA while FcγRIIB mRNA expression was upregulated and this coincided with a decreased phagocytosing and killing ability. Those effects were correlated with the levels of TNF-α. The abovementioned mechanisms may contribute to understand how secondary bacterial agents affect PRRSV-infected pigs.

In summary, virulence and immunity in PRRSV infection are two sides of the same history. Thirty years after the emergence of the first cases of the disease we start to understand the nature of this infection.

Multidrug-resistant s*almonella*: a threat to food safety and public health

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Salmonella is an important cause of foodborne infections in humans; globally, an estimated 93.8 million cases of salmonellosis are reported each year, resulting in 155,000 deaths. Multidrug-resistant (MDR) *Salmonella* have been reported worldwide. Infections with such *Salmonella* are associated with increased morbidity and mortality. Common serotypes pathogenic to both humans and swine include *S. typhimurium, S. enteritidis, S. agona* and *S. heidelberg*. *S. choleraesuis* and *S. typhisuis* are host-adapted to swine but can also cause human illness. *Salmonella* infections in asymptomatic swine may serve as a source of human salmonellosis via contamination of pork products. The use of antibiotics in food animals has contributed to the emergence and dissemination of antibiotic-resistant pathogens. Many *Salmonella* have developed resistance to antibiotics used to treat severe infections, including ceftriaxone, ciprofloxacin, and ampicillin. A better understanding of resistance mechanisms and resistance gene transfer is critical to develop control measures for this public health issue.

Designing novel vaccines against porcine reproductive and respiratory syndrome virus

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Porcine reproductive and respiratory syndrome virus (PRRSV), isolated more than 30 years ago, is arguably the most economically-important global swine pathogen. Despite intensive research and development efforts, PRRS remains difficult to control causing more than $600 million to swine producers per year in the United States alone. The current available commercial vaccines are mostly modified live-attenuated vaccines (MLVs) based on a single virus strain. The available vaccines are generally effective against homologous or closely-related strains but are ineffective against heterologous strains. Therefore, novel vaccines that confer broad heterologous protection against the genetically-diversified field strains of PRRSV are needed to effectively control PRRS. We have recently employed a number of novel vaccine design approaches for developing novel PRRSV vaccines, which will be discussed in this talk. We demonstrated that molecular breeding through DNA shuffling of PRRSV structural genes of multiple genetically-distinct PRRSV strains produced chimeric viruses as candidate MLVs with broadly-protective ability against heterologous strains. Furthermore, we showed that *in vivo* targeting of shuffled PRRSV antigen as subunit vaccine through porcine DC-SIGN molecule to dendritic cells elicits CD4 T cell immunity in pigs. We also showed that recombinant PRRSV MLVs expressing membrane-bound cytokines as immunomodulatory adjuvants enhances NK and γδ T cell responses and confers heterologous protection against PRRSV. By using the synthetic attenuated virus engineering (SAVE) technology, we codon-pair de-optimized the GP5 gene of PRRSV to rapidly attenuate PRRSV for rapid production of farm-specific killed vaccines. We have also identified critical amino acid residues that are important for PRRSV quasispecies diversity in PRRSV RdRp, which will aid in future design of improved MLVs with high replication fidelity.

Emerging and zoonotic viruses in swine: swine hepatitis E virus as an example

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Emerging and re-emerging viruses pose a constant threat to human and veterinary public health worldwide. Numerous viruses have recently emerged or re-emerged in the global swine population, and can be generally grouped into three broad categories: (1). those infecting only pigs causing economically-important diseases such as porcine epidemic diarrhea virus (PEDV), porcine reproductive and respiratory syndrome virus (PRRSV), and porcine circovirus type 2 (PCV2); (2). those zoonotic viruses that can be transmitted from pigs to humans with or without causing diseases in pigs such as swine hepatitis E virus (swine HEV), Nipah virus (NiV), and H1N1 pandemic influenza virus; and (3). those infecting pigs but with uncertain or unknown clinical implications such as Torque teno sus virus (TTSuV), and porcine lymphotropic herpesviruses (PLHV). Here in this talk, by using swine HEV as an example, the zoonotic risk and pork safety associated with emerging and zoonotic swine viruses are discussed. Swine HEV infection is widespread in the global swine population. The genotypes 3 and 4 swine HEV in pigs can readily cross species barrier and infect humans causing hepatitis E. Pig handlers such as swine veterinarians and producers are at higher risk of zoonotic infection by swine HEV. Pork or pork products from the grocery stores are contaminated by swine HEV, and sporadic and cluster cases of hepatitis E have been definitively linked to the consumption of raw or undercooked pork or pork products (such as sausages). The majority of the zoonotic genotype 3 HEV-infected immunocompromised individuals such as organ transplant recipients progresses into chronicity. Understanding the ecology, natural history, and mechanism of cross-species infection of zoonotic swine viruses will help devise effective preventive and control strategies against them.

Changing siglec-usage of PRRSV to enter macrophages, the basis of differences in PRRSV virulence/pathogenicity and evolution?

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Keywords: PRRSV, pathogenesis, virulence, pathogenicity, receptors, evolution

Porcine reproductive and respiratory syndrome virus (PRRSV) is a genetically unstable arterivirus that is causing devastating infections in swine all over the world. Although the virus only started to cause overt disease since the late eighties, the virus has been circulating and evolving during a much longer time. The fact that PRRSV is evading several branches of the immunity is a clear proof of the long virus-host co-evolution. The reason why the virus caused at a certain moment clinical signs and later on even increased its virulence and pathogenicity may be found in its specific macrophage tropism and the possibility to switch to new binding receptors (siglecs), allowing the virus to use and kill different macrophage subpopulations. This may explain the different virulence and pathogenicity characteristics.

In the past, several entry mediators have been described. Siglecs (sialic acid-binding immunoglobulin-type lectins) have been demonstrated to function as binding and internalization receptors, shuttling PRRSV inside the macrophage. The heterocomplex GP5/M was identified as viral ligand. Once inside the cell, CD163 is responsible for the disassembly, releasing the genome in the cytoplasma, leading to the transcription of the polyproteins and the formation of the replication/transcription complex. The GP2/3/4 complex is the viral counterpart of CD163. In the past, our group demonstrated that Lelystad virus is using sialoadhesin (= siglec-1) for entering a large subgroup of macrophages that can be found all over the body, but is strongly concentrated in the lungs (intravascular, perivascular-interstitial and alveolar macrophages), endometrium/placenta complex and to a lower degree in lymphoid tissues. This could clearly be correlated with the problems that were observed in the field: respiratory and reproductive problems. Several recent European strains were more virulent and were replicating to high levels in the nose, which was not the case for LV. When looking in-depth into the phenotype of the infected macrophage, a high percentage appeared to be sialoadhesin negative. We hypothesized that the virus used another siglec to enter the nasal macrophages. In the quest to find the alternative siglec (s), we cloned siglec-3, -5 and -10 and expressed them in PK-15 cells together with the disassembly receptor. Siglec-10 was demonstrated to function as a PRRSV receptor for both type 1 and 2 strains, but the type 2 strain was growing better. By recent comparison of the growth of several type 1 strains and type 2 strains in PK15 siglec1^{+}CD163^{+} and PK15 siglec10^{+}CD163^{+} cells, it could be concluded that all PRRSV strains are using both receptors, but with a different power and that the replication of PRRSV upon entry via siglec-10 was giving a much better virus yield per infected cell than via siglec-1. Siglec-1 was preferentially used by type 1 strains (both subtype 1 and subtype 3), whereas type 2 strains were preferring siglec-10. In addition, there was a clear correlation between the growth power of the virus in the cells independent of the receptor type and the clinical background. Strains that were typed as being avirulent showed a very restricted replication in both cell lines. Aggressive strains replicated extremely fast reaching high virus titers. In the pig, siglec-10 is expressed at low levels in a certain subset of macrophages in lymphoid tissues. Because it is also expressed to high levels in B-lymphocytes, we believe that PRRSV may be taken up and affect their function, in part explaining the immunosuppression caused by PRRSV. Research is ongoing to analyze this issue. Because siglec-10 positive macrophages were not found in the nasal mucosa, we believe that there is still another very important siglec-x expressed in the nasal macrophages, which is related with the virulence/pathogenicity of the PRRSV strain.

Based on the obtained published and unpublished results, we believe that PRRSV has glided over different siglec receptors in its evolution. It most probably started with siglec-10 allowing the virus to replicate in tonsils as entry organ and internal lymphoid tissues as secondary replication sites. This may explain why replication via this receptor gives the highest yield. This most probably did not lead to big problems (slight immune suppression), allowing the virus to replicate

and spread without recognition by the farmer/veterinarian. There are still regions in Eastern Europe, where such strains are circulating. Only when the virus switched to siglec-1, lung and placenta macrophages became infected, causing overt respiratory and reproductive problems. When finally, the virus started to use in addition siglec-x, the virus replicated to high levels in nasal macrophages (and most probably also in other macrophages, such as microglia cells, causing replication in CNS and leading to central nervous disorders, and perivascular macrophages, causing effusion (exudate in body cavities), leading to the most devastating clinical outcome.

Prevention and control of swine diseases through biosecurity

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Introduction

Prevention and control of swine diseases have to be comprehensive approach, which should include the components as below:

1) Pig flow
2) Herd immunity (vaccination, etc.)
3) Medication
4) Husbandry / management
5) Testing
6) Biosecurity

The objective of this paper is to focus on the importance of biosecurity in particular. Topics we discuss are as follows:

- What is biosecurity?
- How is an infectious agent transmitted?
- What can we do to reduce a risk of each transmission route?
- How can we audit and measure biosecurity?
- Conclusions & summary

What is biosecurity?

Definition of biosecurity is “the protection/security of susceptible animal herds from the introduction and transmission of infectious pathogens” (*Saunder’s Veterinary Dictionary, 1999*).

Biosecurity has to be:

1) Science based
2) Practically feasible (simple, organized)
3) Effective (cost vs. benefit)
4) Committed to continue (execution)
5) Measurable

Components of biosecurity include as follows:

1) Internal biosecurity (within-farm)
- To minimize the transmission of pathogens that already exist within a farm

2) External biosecurity (Between-farms)
- To prevent new introduction of pathogens into a farm

3) Monitoring, auditing, and education

Transmission routes of infectious agents

Transmission routes of infectious agents are classified as below:

1) Direct transmission (porcine vectors)
- Live animals
- Semen

2) Indirect transmission (non-porcine vectors)
- Needles

- Personnel
- Coverall and boots
- Fomites
- Transport
- Carcass disposal
- Birds
- Rodents
- Wild animals
- Insects
- Manure processing
- Water
- Air
- Feed

Measure biosecurity risks on your farms

Biosecurity should be numerically measurable. Here are some examples of tools that are able to asses biosecurity risks on farms:

- PADRAP (AASV: American Association of Swine Veterinarians, North America) (1)
- BioCHECK (Ghent University, EU) (2)
- BioAsseT (P-JET: PRRS-Japan Elimination Team, Japan) (3)

Transboundary risk of swine disease transmission

Most recently, Dee et. al. has developed a transboundary model to prove the risk of transmission of certain swine pathogens such as PEDV, PRRSV, Seneca Valley virus (as a surrogate of FMDV) and ASFV via selected feed ingredients (4).

Area regional approach and global collaboration

In some regions, economical significance of particular swine diseases such as PRRS and PED has let producers and veterinarians to initiate area regional approach in order to control or eliminate such diseases. Recent research has shown that a risk of transmission of certain swine pathogens is transboundary (4). Global collaboration is required for sustainable success of biosecurity in each country.

Conclusions & summary

Biosecurity is an only way of the “true” proactive approach of disease prevention. Biosecurity should be comprehensive approach. Execution is the key of prevention/control of swine diseases through successful biosecurity. Because the risk of swine disease transmission is transboundary, sustainable success of biosecurity requires area regional approach and global collaboration.

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Models for understanding the genetics of the host response to PRRSV infection

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In 2007, the National Pork Board (NPB) along with USDA and members of the scientific community formed the PRRS Host Genetics Consortium (PHGC). The goal of the PHGC is to identify genomic markers that lessen the impact of PRRS on the commercial pig industry. The standard PRRS model developed by the PHGC includes groups of 200 nursery pigs infected with well-characterized PRRSV isolates. The piglets are usually followed for 42 days after infection; a period covering the acute phase of infection and early onset of persistence (defined as the disappearance of virus from the blood, but still present in peripheral tissues). Piglets and parents are genotyped for >60,000 single-nucleotide polymorphisms (SNPs) using the Porcine SNP60 Bead Chip. PRRSV-related disease traits, or phenotypes, include virus load, weight gain, anti-nucleocapsid (N) antibody response, virus neutralizing titer, morbidity, and mortality. Deeper phenotyping approaches include RNA-Seq of whole blood and tonsil RNA. The nursery pig model was used to identify a marker on *Sus scrofa* chromosome (SSC) 4, which is associated with decreased virus load and improved weight gain. The SSC 4 marker, WUR10000125 (WUR), locates to a 1.0 Mb region occupied by members of the guanylate binding protein (GBP) family of interferon-inducible genes. Resistance to PRRS is associated with the expression of wild type GBP5, which is anchored to the Golgi and is predicted to affect PRRSV infection at several levels. The WUR marker is being utilized by genetic companies. A second infection model is an extension of the nursery pig model to the field. Groups of 200 nursery pigs are placed in pig barns that are disease-challenged, including the presence of endemic PRRS. Pigs are followed all the way to market. The results show a favorable response of pigs possessing the WUR marker. A third model, designed to reproduce PRRSV-associated disease, incorporates co-infection of pigs with PRRSV and PCV2. This model replicates many of the characteristics of PCVAD. One marker linked with PCV2 infection is found on SSC 7. The experimental or natural infection of almost 5,000 pigs has resulted in an improved understanding of the dynamics of PRRSV infection at the population level. The identification of genomic markers linked with specific disease traits is now being used by the swine industry.

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Current challenges of porcine circovirus 2 prevention and control

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Introduction

Porcine circovirus 2-systemic disease (PCV-2-SD) is a multifactorial disease that can be controlled by the use of PCV-2 vaccines. Prior to the advent of vaccines, prevention and control was focused on eliminating environmental and infectious co-factors believed to trigger the disease. Since mid-2000s, commercial PCV-2 vaccines have been available and economic losses attributed to porcine circovirus diseases (PCVDs), caused mainly by PCV-2-SD and PCV-2-subclinical infection, have been markedly reduced. Importantly, since co-infections are usually present in PCV-2-SD cases, vaccination against PCV-2 has significantly helped in the control of clinical disease associated with polymicrobial infections as well. Most current vaccines worldwide are based on PCV-2a strains. Although vaccines are effective in reducing clinical signs of PCV-2-SD independently of the infecting genotype, their efficacy against mixed infection of PCV-2a and PCV-2b and the recently emerged PCV-2d might be less clear.

Who should be vaccinated?

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 are either 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.

Conclusion

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.

Emergence of mutants of porcine epidemic diarrhea viruses (PEDV) in Korea and application of nanobiotechnology for vaccine adjuvant against PEDV

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Porcine epidemic diarrhea (PED) virus was first reported in South Korea in 1992 and has become one of the most important porcine diarrhea associated viruses in South Korea. We per-formed large-scale study of the incidence of PEDV in pigs with diarrhea in South Korea, and consequently identified and characterized a novel PEDV variant with large genomic deletion. A total of 2,634 fecal and intestinal samples were collected from pigs exhibiting diarrhea from 569 swine farms in all 9 provinces of South Korea. To determine complete spike (S) gene sequence, we purified, cloned, and sequenced PCR products on an automated DNA sequence by using T7, SP6 primers, and newly designed S gene–specific primers. Of the 2,634 samples, 205 were positive for PEDV. However, when SF2/SR2 primers were subjected to PCR, a strong and single band of unexpected size (≈1,000 bp) was found in each PCR product from3 diarrhea samples of the suckling pigs. Exact length of the band was 981 nt, and the band was much shorter than that of intact fragment because of 612 nt deletion in S gene, corresponding to a 204 aa deletion. The coronavirus S protein plays a pivotal role in regulating interactions with specific host cell receptor glycoproteins to mediate viral entry and stimulate induction of neutralizing antibodies in the natural host. Mutations or deletions in the coronavirus S gene affect its pathogenicity and tissue tropism. Porcine respiratory coronavirus (PRCV), a naturally occurring deletion mutant of transmissible gastroenteritis virus (TGEV), is an example of pathogenic change and tropism switching, apparently associated with S gene change. These amino acid mutations of PEDV might cause the conformational change of S protein and result in antigenicity/immunogenicity alteration of the PEDV variant.

The field of nanotechnology encompasses those technologies to fabricate materials, including sphere, cubic and nanoscale particles. Therefore, nanobiotechnology has the potential to offer not merely advances in diagnostics and vaccination to control infectious diseases. Herein, we also address nanocomplex of amphiphilic grafted poly (amino acid) and hydrophobic squalene (PA/S-NC) as a potent adjuvant that can act as a robust strategy for induce humoral (Th2)and cellular (Th1) immune responses as well as a delivery agent of antigens. We made PED vaccine with polymer based vaccine adjuvant system and evaluated its immunogenicity by ELISA assay in guinea pigs. The results in this study showed that the synthesized adjuvant presented enhanced immunogenicity *in vivo*, and maintained cell viability *in vitro*. Specifically, the 3rd inoculation lead notably high IgG values in guinea pigs. However, this requires further efficacy study in targeting animal model: pigs.

Consequently, this vaccine adjuvant presented improved immunization efficiency, and could be a promising vaccine adjuvant candidate for PED vaccine.

PRRSV epidemiology: emergence of new virus strains

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Keywords: genetic diversity, sequencing, virus emergence, epidemiology

Porcine reproductive and respiratory syndrome (PRRS) is a globally distributed viral disease clinically characterized by reproductive failure in breeding animals and respiratory disease in pigs of all ages. The disease is caused by PRRSV-1 and PRRSV-2, the two virus species currently classified within genus *Porarterivirus*, family *Arteriviridae* (https://talk.ictvonline.org/taxonomy/). Both PRRSV species are worldwide distributed and exhibit significant genetic and antigenic. PRRSV-1 is genetically more diverse than PRRSV-2, but the most widely globally distributed PRRSV-1 strains belong to a single genetic subtype 1, while subtypes 2, 3 and 4 were discovered only in Eastern Europe and Russian Federation. However, unlike in the case of PRRSV-2, relatively few complete genome sequences of PRRSV-1 are known, and the classification into genetic subtypes is largely based on the analysis of ORF5 and ORF7. Thus, the true level of genetic diversity within PRRSV-1 and its biological importance remains unclear.

PRRSV diversification in a given area is generated through several mechanisms. It is generally accepted that the species of PRRSV evolved in isolated pig population over long time. The most common recent ancestor of PRRSV-1 and PRRSV-2 is thought to exist around 1880. Since then, viruses of both species were distributed nearly globally, and were discovered only in the late 1980-ties and early 1990-ties. The evolution PRRSV-1 genetic subtypes was likely a long term and multisite process, before the spread of these viruses across vast areas of Eastern European and Asian parts of the former Soviet Union. The currently available sequences place the most recent common ancestor (TMRCA) of PRRSV-1 strains between 1946-1967, ie, during the post-World War II development of Europe. Molecular clock calculation dated TMRCA of Western European PRRSV-1, subtype 1 viruses only, in 1979, 10 years before the disease was first observed. However, these estimations are based on the analysis of rather limited genetic data.

The lack of known natural reservoirs and vectors of PRRS viruses, other than suide, able to transport them over long distances, provides strong evidence that the trade live pigs or semen, was, and still is the main factor of long distance transmissions of the virus, or its variants “exotic” for a given area. Locally, wild boar can play a role in transmission of foreign PRRSV strains, as it was showed in Lithuania. Obviously, countries, or regions receiving live pigs from multiple sources are at highest risk of emergence of novel variants. The risk is highest during periods of rapid development of pig farming, or changes in production profile. A good example comes from Central Eastern European countries, such us Czech Republic, Hungary, Poland, Romania or Slovakia, and others. Most of these countries were under the domination of the former Soviet Union from 1940-ties and their close co-operation was in frame of COMECON treaty until the beginning of 1990-ties, so at the time when PRRSV-1 viruses were already diversified into distinct genetic subtypes. Despite close economic and political links between Soviet Union and other countries of the Communist Block, genetic diversity of PRRSV-1 at the time of its discovery in the regions, was very different. A sharp geographical demarcation line of PRRSV-1 diversity pattern in Europe, was found along the western borders of the former Soviet Union (with Poland, Slovakia, Hungary and Romania). West of the border only subtype 1 viruses were detected. One explanation of why genetic subtypes 2, 3 and 4 were never found outside the of the former Soviet Union states can be that it was a major importer of pigs and pig, also from countries of the Communist Block. On the other hand, rapid changes in pig production after the collapse of socialist rule in the beginning of 1990-ties, involved closer links with Western European countries, and import of live pigs. This could explain why genetic diversity of PRRSV-1 in Central Eastern European countries is similar to Western European countries, and different from Eastern Europe and Russia.

Another factor that contributes to PRRSV diversification is the use of modified live vaccines against PRRS. Vaccines

based on either PRRSV-1 or PRRSV-2 attenuated viruses, if not used with care, can lead to vaccine related virus persistence in a farm and eventually spread to non-vaccinated population. The spread of PRRSV-2 vaccine related viruses in a population where wild type variants belong to PRRSV-1, as in Europe, is relatively easy to detect with differential PCR. On the other hand the spread of vaccine related viruses in a population infected with wild type viruses of the same PRRSV species requires nucleotide sequencing, and even then the results of the analysis are sometimes difficult to interpret due to the fact that wild type viruses highly similar to vaccine parental strains may still circulate.

Two or more PRRSV strains from one species can recombine if they co-infect the same cell. Recombinants of PRRSV-1 and PRRSV-2 strains were never detected. Recently Renson et al. (2017) identified a recombinant of two PRRSV-1 modified live vaccine viruses which were applied in the same pig population. The proportion of recombinant strains circulating in pigs is very difficult to assess because recombination can occur in any part of the genome while most of the PRRSV genetic data generated for diagnostic or epidemiological purposes come from ORF5 and sometimes from ORF7. Obviously, it restricts chances to detect genetic recombination only to cases involving 3' end of the genome. There are multiple examples of the detection of recombination events after analysis of complete genomes but, very likely the recombination between closely related strains can be difficult, or impossible to detect. The importance of genetic recombination for virulence of such new strains is difficult to assess. Nevertheless, it poses a serious problem for molecular epidemiology as the analysis of different genomic fragments of a given strain can give contradicting results regarding its origin or relationship.

The most widely used methods for PRRS diagnosis include ELISA, PCR and nucleotide sequencing. Most, but not all ELISA kits universally detect seroconversion induced following contact with PRRSV-1 and PRRSV-2, with similar sensitivity. However, due to broad cross reaction ELISA does not allow for ultimate discrimination between PRRSV-1 and PRRSV-2 seroconversion. There are many commercial and in house PCR assays being used in diagnostic laboratories, and some of them allow for discrimination between PRRSV-1 and PRRSV-2, what is very important considering worldwide distribution of both species. However, very high genetic diversity of PRRSV-1 and PRRSV-2 makes the production of globally universal and robust kits very difficult. The design of primers and probes to detect PRRSV-1 strains circulating in Eastern Europe and Russian Federation is particularly challenging due to their extreme diversity and a very few sequences available. Another problem of PCR diagnosis of PRRS is a certain continental bias. In Europe, PRRSV-2 is considered to be genetically homogeneous and non-pathogenic (with exception of Denmark). The detection of a new lineage of PRRSV-2 in Hungary, Slovakia and Romania provided the first strong evidence that the genetic diversity of this PRRSV species in Europe can be higher than it was previously believed. It raises a question whether the available diagnostic PCR assays (commercial and in house) used in Europe are properly validated to be able to detect broad range of PRRSV-2 strains. The situation in Americas and Asia where PRRSV-2 dominates is opposite, and PRRSV-1 is sometimes neglected. This bias results in some level of uncertainty about the role of either species of PRRSV in PRRS signs and related economic losses, and PRRSV epidemiology.

In 1996 in several states of the USA unusually acute PRRS outbreaks were observed. These outbreaks were characterized by an elevated abortion rate as well as sow mortality and occurred in farms vaccinated with modified live vaccines against PRRS. PRRSV-2 viruses isolated from these outbreaks were genetically different from vaccine strains, but also different from each other. Most likely factors other than the PRRSV strains themselves, such as changes in swine management practices or the presence of another pathogen played role in the emergence of these acute outbreaks of PRRS.

A few years later, in 2001, a virulent variant of PRRSV-2 (MN184) was identified in the US state of Minnesota. In this case all isolates were genetically closely related and characterized by three discontinuous deletions in the *nsp2* region of the replicase ORF. The origin of this variant was never exposed but it was suspected to be in Canada where similar viruses previously existed, and from where high numbers of weaned pigs were transported to Minnesota.

In China in 2006 highly pathogenic PRRS appeared and quickly spread to many provinces causing huge losses to the country's swine industry. Phylogenetic reconstructions indicated that the pathogenic PRRSV-2 variant actually evolved from a local, Chinese PRRSV-2 strain. The highly pathogenic variant had deletions in *nsp2* region of the genome.

However, later studies excluded the *nsp2* deletions as determinants of high virulence.

PRRSV-1 strains are believed to be less virulent than PRRSV-2 viruses. However, most of the observations regarded subtype 1 which is globally spread. Clinical reports from countries where other genetic subtypes (2, 3, 4) are common (e.g. Russian Federation, Ukraine, Belarus), are largely missing or are incomplete. The unique character of these variants attracted attention of researchers in Europe and several reports of challenge experiments emerged. Until now five strains of subtype 2 and 3 from Belarus and Russia were characterized in vivo and four of them were shown to be significantly more virulent than control strains from subtype 1.

More recently PRRSV-1 strains from subtype 1 were reported from Western Europe. In 2013 in some Belgian farms uncommon long-lasting anorexia, fever and respiratory problems within the first two weeks after weaning were observed. Challenge experiments showed relatively severe disease and high viremia induced by two strains isolated from cases of acute PRRS. However, they were only distantly related genetically. In 2014 in Italy a pig farm experienced uncommonly severe PRRS outbreak characterized by high fever, severe respiratory and systemic clinical signs and high post-weaning mortality (up to 50%). Challenge experiment with the PRRSV-1 subtype 1 isolate from the case farm performed in conventional 4 weeks old pigs showed it's high pathogenicity expressed as high fever, dyspnoea, severe lymphocyte depletion in lymph nodes and thymus as well high viremia. Unusually severe PRRS caused by PRRSV-1 subtype 1 virus was observed also in Austria in 2015.

In summary, unusually pathogenic PRRSV strains occur in every geographic location and can be caused by both PRRSV species or genetic subtypes know to date. However, clinical picture observed in the field is always an effect of several factors (farm management, environmental conditions, existing infections, feeding etc.), besides the characteristics of a given PRRSV strain. Also, the outcome of challenge experiments with the same strain can be different. Passage history, health status, age and genetics of pigs used, can have impact on the clinical signs and lesions observed following the infection in experimental conditions. Unfortunately, universal markers of PRRSV virulence are not yet established.

Improving pig welfare and meeting emerging challenges

Peter Stevenson
Compassion in World Farming

Retailers and chain restaurants are demanding higher welfare standards. The global pig sector faces six major welfare challenges. First, producers need to move to group housing of pregnant sows. Aggression in group housing can be avoided with skilful design and management. Second, farrowing crates should be replaced with free farrowing systems. In the best of these piglet mortality can be kept as low as in crates. Third, castration must be brought to an end. Instead farmers should use immunocastration or rear entire males. Fourth, effective enrichment materials should be provided. Fifth, routine tail docking should be ended. Farmers should only tail dock if they have first tried to prevent tail biting by improving the conditions in which the pigs are kept but nonetheless still have a tail biting problem. Sixth, we need to end the use of antimicrobials as growth promoters and for routine disease prevention. Disease should be prevented by good husbandry, housing and hygiene rather than by routine recourse to antimicrobials. In addition, concepts of animal welfare are changing from a focus on the prevention of suffering to also recognising the need for animals to have positive experiences.

Intensive pig production's huge demand for cereals and soy as feed has fuelled the intensification of crop production which, with its monocultures and agro-chemicals, has fuelled overuse and pollution of water, soil degradation and biodiversity loss. High consumption levels of red (including pork) and processed meat are contributing to heart disease and certain cancers. Current diets with their high levels of meat will make it very difficult to meet the Paris climate targets. The pig sector needs to reformulate itself; it needs to become a provider of quality pigmeat rather than the supplier of a cheap bulk commodity.

Keywords: welfare, group housing, farrowing, castration, tail docking

Re-emerged pseudorabies virus: what has been changed?

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Pseudorabies, caused by a herpesvirus, Pseudorabies virus (PRV), has been eradicated in many countries by using gene deleted vaccines. Since 2011, however, a disease characterized by neurologic symptoms and a high number of deaths among newborn piglets has occurred among Bartha-K61–vaccinated pigs on many farms in China and many pig farms with gE antibody negative were turned positive up to 70% in a few months. The causative agent of the diseases was soon identified as pseudorabies virus [1]. PRV is a DNA virus containing approximately 150Kb genome, and only one serotype was known till now. For the re-emerged PRV, what has been changed so that the virus could break through immunization defense established by conventional vaccination? The genomic sequence comparison showed that the re-emerged PRVs isolated from vaccinated pigs displayed in a new branch which distinct from vaccine viruses, and animal infection experiments proved that the emergent PRV could have been substantially changed in aspects of immunogenicity or pathogenicity.

Genomic sequence comparison:
To identify genetic characteristics of PRV strains, we obtained the genomic sequences of emergent PRV strains JS-2012 and HeN1, which were compared to 4 PRV genomes and 729 partial gene sequences. PRV strains isolated in China showed marked sequence divergence compared to European and American strains. Phylogenetic analysis revealed that for the first time PRV can be divided into 2 distinct clusters, with Chinese strains being genotype II and PRVs isolated from other countries being genotype I. Restriction fragment length polymorphism analysis confirmed differences between HeN1 and Bartha strains, as did the presence of unique insertion/deletion polymorphisms and microsatellites. This divergence between the two genotypes may have been generated from long-term, independent evolution, which could also explain the low efficacy of the Bartha vaccine in protecting pigs infected with genotype II PRV [2].

Pathogenicity determination:
In this study, the pathogenicity of the PRV variant JS-2012 strain to pigs was investigated by experimentally inoculating piglets of different ages in comparison with a classic virulent PRV SC strain. The JS-2012 strain caused an earlier onset of clinical signs and higher mortality in 15, 30, and 60-day-old pigs, as compared with a classic virulent PRV SC strain. The Bartha-K61 vaccination provided complete protection against challenge with classical virulent PRV, but only partial protection against challenge with the JS-2012 strain in piglets. In conclusion, the increased virulence of the PRV variant may have partly contributed to the PR outbreak in China [3].

Determination of protective Immunogenicity:
Glycoprotein B was known to be a major protective antigen protein of PRV. Sequence analysis demonstrated that the gB gene of the emergent PRV variant JS-2012 had multiple variations compared with the vaccine strain Bartha-K61. Therefore, we speculate that Variations in glycoprotein B may contribute to immunogenic difference between PRV variant JS-2012 and Bartha-K61. In the study, a specific CRISPR/Cas9 system combined with homologous recombination was used to construct two recombinant viruses, BJB (Bartha-K61 +JS-2012gB) and JBJ (JS-2012-ΔgE/gI +Bartha-K61gB), by interchanging the full-length gB genes between Bartha-K61 and JS-2012-ΔgE/gI. The two recombinant viruses showed similar characteristics in growth kinetics in vitro and similar pathogenicity in mice, as compared to their parental strains. Immunization of mice with inactivated BJB or JBJ followed by challenge of JS-2012 showed that BJB could increase protective efficacy to 80%, compared to only 40% protection by the parental Bartha-K61 strain. JBJ had a decreased protective efficacy of 65%, as compared to 90% protection by its parental JS-2012-ΔgE/gI strain. Exchange of the gB

gene markedly altered the immunogenicity of the recombinant PRV. These data suggest that variations in gB might play an important role in the poor cross-protective efficiency to the emergent PRV variant [4].

Cross-protection between the emergent PRV and classic PRV:

The commercially available PRV vaccine could provide full protection to the classical PRV challenge, but poor protection against the PRV variant. In this study, a gE/gI deleted PRV strain JS-2012-△gE/gI was generated from a PRV variant strain using homologous DNA recombination. Compared to the parental strain JS-2012, JS-2012-△gE/gI grew slowly and showed small plaque morphology on Vero cells. The safety and immunological efficacy of JS-2012-△gE/gI was evaluated as a vaccine candidate. JS-2012-△gE/gI was avirulent to suckling piglets, but was able to provide full protection for young piglets against challenge with both the classical virulent PRV and the emergent PRV variant. After sows were vaccinated with the gE/gI-deleted strain, their suckling offspring were resistant to an otherwise lethal challenge with the classical or the variant PRVs [5].

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Transmission of influenza A virus in pigs: the role of the piglet

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Keywords: influenza, transmission, swine, piglets

Introduction

Influenza A virus (IAV) remains one of the most important respiratory infectious diseases in humans and animals. In swine, IAV causes respiratory disease characterized by anorexia, fever, sneezing, coughing, rhinorrhea and lethargy and the febrile state in pregnant animals can lead to abortions. The disease is characterized by low mortality but high morbidity and decreased growth performance which results in increased pig weight variation. Besides the effects on animal health, IAV is an important zoonotic pathogen and pigs can be a reservoir and a source of novel reassortant viruses, including viruses of pandemic potential. Therefore IAV has implications for both animal and public health, and understanding transmission of IAV in pigs is crucial to prevent zoonotic infections and mitigate the economic impact of the disease to swine farmers.

Transmission of IAV is complex to say the least. Influenza virus was first recognized as a viral agent causing respiratory disease in pigs in 1918. For many years, swine influenza viruses in the USA remained relatively stable until 1998. Subsequently, new strains, new subtypes and multiple reassortant viruses have been identified in pigs in North America. The new reassortant viruses contained genetic components derived from both human and avian species, which resulted in new strains that are difficult to control. Unfortunately, the detection of novel strains continues today, and with it the challenges to control influenza.

As the economic impact of influenza in pigs is recognized and quantified, there is a growing need to design control programs not only directed at mitigating the impact of the disease, but also at minimizing the risk of transmission with the eventual elimination of the virus. Unfortunately, we lack information and defined protocols to achieve sustainable elimination, and in part, the challenge is two-fold. In one end, we have the on-going introduction of strains into herds via people or pigs or other sources poorly studied, and in the other end, there is the persistence of endemic infections due to the on-going infection of susceptible individuals.

Swine production systems have changed significantly during the last 20 years and most pigs today are reared in well circumscribed populations. Infection and transmission dynamics in large populations can differ significantly from the dynamics observed at the individual animal level or in small groups. In individual pigs, flu infections are self-limiting with an average duration of infection of 5 to 7 days. In contrast, flu infections in populations can be maintained for longer periods of time ranging from weeks to years. There are many factors (known or suspected) that contribute to the maintenance of influenza infections in populations including the infusion of animals, varying levels of immunity, and the various routes of virus spread within populations. However, how these factors interact to affect virus introduction and virus maintenance is not well understood or well known.

Transmission of influenza in pigs

The general routes of influenza virus transmission include aerosol, large droplet, and direct contact with secretions of infected individuals or contaminated fomites. Influenza virus transmission through direct contact with infected pigs is considered the major transmission route. Both sick and subclinically infected pigs likely play a large role in the transmission of influenza virus within and between swine herds, highlighting the importance of controlled animal movement and practices to minimize the transmission of infectious agents. Influenza virus in pigs is not transmitted through semen.

Indirect transmission of influenza virus is also assumed to take place in field settings. Water contaminated with bird feces

has been implicated as a source of influenza virus in several swine outbreaks involving avian origin viruses. Routes of infection in wild pigs are not well known but exposure is likely related to contact with either wild bird droppings, contaminated water or access to commercial pigs. Transmission of influenza virus via other indirect routes such as aerosols and fomites have not been studied extensively in pigs.

Influenza virus has been detected or isolated in air samples from rooms of experimentally infected pigs, in the exhaust air from infected farms, in air samples collected at one mile from an infected farm, in the indoor air of clinically affected farms throughout the course of acute infections and in the air of a swine live animal market highlighting the potential role of aerosols in the transmission between pigs, farms and from pigs to people. Pig farm proximity to turkey flocks has also been associated with turkey flock seropositivity to swine-origin influenza virus. In humans, mathematical models have suggested that the airborne route may be the dominant route of influenza transmission.

Transmission via contaminated fomites also plays a role in the spread of influenza virus. Allerson et al., showed transmission of influenza virus between an infected population and a population of naïve pigs when study personnel moved between rooms with and without following standard biosecurity procedures (i.e hand sanitation, change of coveralls and change of boots). However, IAV transmission via fomites under field settings is poorly understood and understudied.

Transmission of influenza virus through pig transport has not been granted much attention until recently. Long distance pig movement has been implicated in the spatial dissemination of influenza viruses of human origin from swine production areas into the Midwest US swine population. Additionally, pig transport has been shown to be responsible for the transfer of infected pigs from breeding herds to weaning sites contributing to the movement of influenza virus between different production sites. Risk of contamination of transport equipment with IAV has not been reported.

One aspect of influenza infections that also contributes to transmission and diversity of influenza viruses in pigs is the introduction of viruses of human origin. Nelson et al., documented 49 human to swine transmission events of pH1N1 and 23 seasonal H1& H3 introductions concluding that humans contribute substantially to the influenza virus diversity found in pigs. Therefore, efforts to decrease the introduction of human origin viruses should also be taken in consideration.

Influenza virus population dynamics, diversity and the role of piglets at weaning in the dissemination of influenza viruses

IAV is considered widespread in pig populations. Very few farms in the USA are considered IAV negative. In a longitudinal active surveillance study, Corzo et al., reported that approximately 90% of herds surveyed tested positive for influenza at least once. Influenza circulation was detected in vaccinated and non-vaccinated herds, in herds with and without clinical signs, and throughout the year showing less seasonality than inferred previously from diagnostic laboratory submissions.

Pigs at weaning are considered a main source of IAV dissemination to other farms. In multi-site production systems, piglets are transported to other farms at weaning and often they are subclinically infected representing “silent spreaders” of IAV although farms can be infected year around. Seasonal patterns can be partially explained by outdoor absolute humidity and temperature trends. About 28% of weaned groups out of 1,523 tested positive for IAV at weaning. Furthermore, co-circulation of distinct influenza viruses is common in piglets which results in co-circulation of strains in nurseries and finishers. Ten genetically distinct clades of contemporary US swine influenza viruses were identified in piglets at weaning in a Midwestern production system with 21% of farms having 3 genetically distinct viruses circulating over time, 18% farms had 2 and 41% had 1 isolate available. Overall, these results point at the fact that piglets at weaning are important contributors in the spread of influenza genetic diversity across geographical regions.

In addition, differences in prevalence of influenza at weaning can result in different patterns of influenza transmission. We documented 4 different patterns of influenza transmission dynamics in the nursery and these were characterized by differences in the number of pigs weaned influenza positive, the duration of the infectious period and recovery, and the transmission rates observed within the groups. We observed 2 patterns with high influenza prevalence at weaning. In these cases most piglets were positive at weaning and infection resolved very quickly. Only in one pattern there was a recurrent infection at approximately 4 weeks. Based on the testing done, this second peak of infection was caused by a

virus similar to the one detected at weaning although we cannot completely rule out the co-circulation of a distinct virus. Groups of pigs weaned with medium prevalence at weaning were also of interest. There were very distinct reproduction ratio values between cohorts of pigs originating from groups with varying prevalence at weaning indicating that transmission parameters were different between groups which may be correlated with the field observation of some strains being endemic and others fading out. Low prevalence cohorts at weaning also appeared to be important from a control standpoint. The fact that there were groups without detectable influenza at weaning suggests that reducing prevalence at weaning below certain level can help reduce the circulation of influenza in the nursery and potentially, preventing the on-ward spread of influenza. Understanding the factors that drive influenza persistence in populations is critical to fully control influenza in pigs.

Immunity can also influence transmission dynamics in populations. Influenza virus transmission has been quantified experimentally in non-vaccinated and vaccinated pigs with a reproduction ratio estimate of 10.66 in non-vaccinated pigs and 1 and 0 for pigs vaccinated with heterologous and homologous inactivated vaccines, respectively. A follow-up transmission study identified a similar reproduction ratio estimate in non-vaccinated pigs and a reduction in transmission parameters in pig populations with homologous maternal immunity. Overall, these studies indicate that immunity may mitigate transmission and reduce the burden of influenza virus in pigs. However, immunity levels are variable in pigs at weaning. There can be significant differences between pigs within a group, and also between batches of pigs overtime. Patterns of immunity as measured by maternally derived antibodies are associated with the transmission patterns described above and the characteristics of the immune response is likely associated with the persistence of endemic infections in piglets.

It is important to note though, that although piglets at weaning play a central role in the maintenance of endemic infections in endemically infected herds and in the dissemination of the virus to other locations, influenza status of replacement animals has also been associated with the introduction of viruses in breeding herds and infections in piglets at weaning. The exact role how these animals alter the landscape of existing virus in herds needs to be further studied.

Summary

In summary, influenza transmission in endemically infected herds is dynamic. Piglets prior to wean represent a major reservoir of endemic flu viruses in breeding herds, but also are major contributors in the dissemination of genetically diverse influenza viruses to other farms, including distant regions. Weaning of infected groups of piglets with different levels of infection and immunity at weaning can result in IAV transmission patterns that may explain the nature of endemic infections, and the fading-out or persistence of infections in growing pigs. Therefore, understanding transmission of influenza viruses will help in the control and elimination of the virus and will mitigate risk of infection to other pigs, other species and to people, reducing the risk of endemic and pandemic infections.

Molecular epidemiology of classical swine fever virus and its control in China

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Since the implementation of nationwide vaccination with C strain, classical swine fever (CSF) has been effectively controlled without large scale outbreak for at least 20 years, but sporadic onset is still present in many regions in China, which is still a heavy burden for Chinese pig industry. The circulation of 4 CSFV subgenotypes (2.1, 2.2, 2.3 and 1.1) was reported between 1990 and 1999 in the country with 2.1 and 2.2 being major subgenotypes. Present study using strains collected between 2000 and 2016 demonstrated that subgenotype 2.1 is predominant, showing significantly genetic diversity than other subgenotypes, which could be divided into at least ten sub-subgenotypes from 2.1a to 2.1j based on phylogenetic analysis using partial (190 nt) or full E2 gene sequences. Except for 2.1d circulating in India, 9 sub-subgenotypes are present in China with some of them closely related to isolates from Europe and other Asian countries. Of these, 2.1b, 2.1c, 2.1h, 2.1i and 2.1j are widely prevalent. To control the disease Chinese government (Ministry of Agriculture) has launched its national plan to start the elimination of CSF in breeding pig farms and selected administrative regions in 2017.

Key words: China; classical swine fever virus; molecular epidemiology; CSF control

Porcine reproductive and respiratory syndrome virus and *Haemophilus parasuis* coinfection enhances inflammatory responses

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Highly pathogenic-porcine reproductive and respiratory syndrome virus (HP-PRRSV) infection causes severe interstitial pneumonia and significantly elevates the levels of IL-1β, IL-10 and TNF-γ. However, the molecular mechanism of inflammatory responses activation induced by PRRSV has not been fully elucidated. Our previous research demonstrated that HP-PRRSV infection activates NLRP3-mediated inflammatory response in primary porcine alveolar macrophages (PAMs). Additionally, PRRSV genomic RNA, 5' untranslated region (UTR), 3' UTR, and nsp7b transcripts of PRRSV genome induce NLRP3 inflammasome activation and IL-1β secretion. We identified host RNA helicase DDX19A as a novel viral RNA sensor. DDX19A binds PRRSV genomic RNA and interacts with NLRP3, which results in the activation of NLRP3 inflammasome in PRRSV-infected PAMs. Our findings provide a deeper knowledge of viral infection–induced NLRP3 inflammation activation and decipher the molecular mechanism involved in initiating recognition of viral RNA to activate inflammatory responses.

We also noticed that HP-PRRSV infection often predisposes pigs to secondary bacterial infection, which results in robust inflammatory responses. However, whether the secondary bacterial infection synergizes HP-PRRSV infection and enhances inflammatory responses is not fully understood. Here, we characterized HP-PRRSV infection-mediated secondary bacterial infection and found that bacterial loads of 11 bacterial species in the lung increased after HP-PRRSV infection, mainly including *Mycoplasma hyorhinis*, *Haemophilus parasuis* (*H. parasuis*) and *Escherichia coli*. The expression and secretion of inflammatory cytokines in PAMs co-infected with HP-PRRSV and *H. parasuis* are increased compared with PAMs infected with HP-PRRSV or *H. parasuis* alone. We also noticed that *H. parasuis* RNA plays an important role in the enhancement of robust inflammatory response in HP-PRRSV and *H. parasuis* co-infected PAMs. Overall, our findings also suggest that bacterial RNA enhanced HP-PRRSV-mediated inflammatory responses, which provides an important clue for comprehensive understanding of HP-PRRSV and bacterial coinfection-mediated pathology.

Nutrient-dependent regulation of health to optimze the reproductive performance of sows in modern swine production

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Nutrients constitute the fundamental basis required for all metabolic activities, and make a great influence not only on the production performance but also the health in all domestic animals. For breeding herds, preventing the reproductive function during a poor health is an adaptive process that is conserved and essential for the survival of species. Besides, health control in the sow herd helps prevent disease spread through other stages of production and is also an important step to create an enhanced immunity for the offspring to counteract the complex environment during their early life. Therefore, it is necessary to optimize the sows health to maximize herd performance, particularly by using any nutritional solutions. The manipulation of nutritional solutions to optimize sow health should be based on the stage of production. For the replacement gilts and the post-weaning sows, nutrients should focus on the hypothalamus-pituitary-gonadal axis to promote the onset of estrus and follicle development, which is critical for the conception and early embryonic survival. For pregnant sows, nutrients could influence the balance between Th1 and Th2 immune response in dams, which provide a suitable uterine environment for the implantation of embryo and placental angiogenesis. In particular, nutrients, like different kinds of dietary fiber, make a great influence on the production of endotoxins in intestine and circulation in sows, resulting in alternations of stress during the late gestation. Finally, the sow milk is the major source of nutrients for suckling piglets, thus the immune status of the suckling piglets were greatly influenced by the immunity of maternal. Nutritional solutions that maximize the sow health could also have a positive effects on the immune function of their offspring. Collectively, optimizing nutritional solutions provide a fundamental basis to enhance the disease resistance and improve the health status of sows.

Keywords: disease resistance; sow; reproductive performance

Current status of PRRS in China: diversified strains of PRRSV and complicated clinical diseases

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Porcine reproductive and respiratory syndrome (PRRS) causes reproductive failure in sows and respiratory diseases as well as secondary bacterial infections in growing pigs. PRRS virus (PRRSV), the causative agent of the disease, is divided into two species: PRRSV 1 (type 1) and 2 (type 2) with characteristics of easily variation and rapid evolution resulting in persistent emergence of diversified strains in phenotype and genotype. PRRS is one of the most economically important swine diseases to the Chinese pig industry, impacting deeply pig production since it was first recognized in the middle of 1990s in China. Especially, the emergence of the highly pathogenic PRRSV (HP-PRRSV) (lineage 8 of PRRSV 2) in 2006, the "devil virus", devastated the Chinese swine industry. While the unexpectedly entry of PRRSV NADC30-like (lineage 1 of PRRSV 2), a visitor from outer space, has aggravated the diversity and divergence of PRRSV in the field. This presentation will focus on recent status of diversity and recombination of PRRSV 2, its leading clinical diseases, and issues regarding PRRSV MLV vaccination, as well as suggestions to more effectively control PRRS in China.

1. The "extinction" of HP-PRRSV and appearance of various HP-PRRSV-like

HP-PRRSV prevailed for several years as a dominating virus in China, leading to inestimable economic losses for pig production. Several MLV vaccines (e.g., JXA1-R, HuN4-F112, and TJM, etc) developed through serial passages of the respective HP-PRRSV strains in MARC-145 cells were commercial subsequently since 2011. These MLV vaccines had been benefited in the reduction of HP-PRRS outbreak and incidence in the field. However, reproductive failures in sows, such as sporadic abortion, stillbirth and weak piglets, and respiratory diseases in growing pigs are very common on pig farms practicing vaccination. Besides themselves safety, massive and multiple vaccinations and unlimited use of the MLV vaccines have raised some concerns: (i) vaccine viruses persist to circulate and evolve on pig herds; (ii) the pathogenicity/virulence reversion of vaccine viruses; (iii) the recombination between vaccine and field viruses. Indeed, several emerged or isolated strains of PRRSV with enhanced virulence have been recognized to be likely revertants of one HP-PRRSV MLV. In recent years, almost real HP-PRRSV could not be monitored from clinical samples of pigs with PRRS manifestations; on the contrary, various HP-PRRSV-like viruses and/or viruses evolved from HP-PRRSV MLV were detected or isolated easily, proposing that HP-PRRSV has been extinct almost in China at the present.

2. The emergence and prevalence of PRRSV NADC30-like

The appearance and wide transmission of virulent PRRSV NADC30-like caused a new round of clinical PRRS outbreak in China since 2014. The exact time when the virus entered to China remain unknown. It was postulated that the virus was introduced by breeding pig from North America. The emerging PRRSV NADC30-like strains is genetically close to NADC30 virus isolated in 2008 and described in 2012 in the United states. The strains all share identical genetic marker to PRRSV MN184 strains and NADC30, namely the characteristic discontinuous deletions in its nsp2-coding region. It was believed that PRRSV NADC30-like prevailing in China was evolved from PRRSV NADC30. PRRSV NADC30-like-infected pig farms all display reproductive failures (10-30% abortion rate in pregnant sows) and respiratory conditions in nursery pigs with less mortality. The NADC30-like has become a dominating virus in recent years, and nowadays, the virus continues to transmit in pig-producing areas in China. Clearly, PRRSV NADC30-like has added further complexity to the PRRSV diversity and PRRS control in the field. Unfortunately, current MLV vaccines can only provide limited cross-protection against NADC30-like infection under experimental condition, and while this less cross-protection is worse in the field. The fact that pig farms with MLV vaccination still suffered the virus infection is a best testimony.

3. Diversity of PRRSV 2 in China

PRRSV 2 is the dominating type in China although PRRSV1 infection can be monitored occasionally from clinical samples. PRRSV 2 can be classified into different lineages due to the genetic divergence of ORF5 gene. Besides lineage 8 and 1 mentioned above, the strains belonging to lineage 3 can be found in south of China, which are epidemic in few pig-producing areas. The lineage 8 inhabits the majority and can further be divided into different sublineages or branches. After the emergence of HP-PRRSV and with the extensive use of several HP-PRRSV MLV vaccines, a tendency of rapid variation and evolution of PRRSV 2 in the field has been recognized. The genetic variations of nsp2 contribute most to the PRRSV diversity. The increasing expansion of nsp2 diversity has been noticed, including various deletions, as long as 150aa and insertions, reflecting the diversity of PRRSV 2 in China. Our latest review summarized at least 23 patterns of nsp2 in the Chinese field isolates. The diversity of PRRSV also exists on pig farms.

4. Continuing generation of recombinant PRRSV

PRRSV has been recognized to share the intrinsic ability to adapt in pigs and evolve in the field. The recombination within the genome is considered to greatly contribute to the evolution and diversity of PRRSV. A number of recombinant strains of PRRSV have been documented in China. The recombination has different types, including the recombination events between MLV and field viruses, MLV-evolved viruses, and inter-lineages (lineage 1 and lineage 8 or lineage 3 and lineage 5, as well as among lineage 1, 3 and 8), and inner-lineages (lineage 8), or both inner-lineage and inter-lineage. Our analyses show that PRRSV NADC30-like has been involved in the tremendous recombinant events, and the virus plays an important role in the generation of recombinant strains of PRRSV in China. Our unpublished data show that, of the four isolates from a single pig farm practicing HP-PRRSV MLV vaccines, one strain is a recombinant virus generated from the recombination event between two HP-PRRSV MLV-evolved viruses, and other one is a recombinant virus between two HP-PRRSV MLV-evolved viruses from one MLV vaccine, and another two strains are recombinant viruses from NADC30-like and a HP-PRRSV MLV-evolved virus. Two strains of HP-PRRSV MLV vaccines were used successively on this farm. Obviously, HP-PRRSV MLVs also contribute to the recombination of PRRSV besides the entry of NADC30-like. Unexceptionally, the recombinant viruses present higher pathogenicity for pigs.

5. Suggestions for PRRS control

Facing the diversity and complexity of PRRSV 2 in China, what do we need to do? We have to think about the issues associated with HP-PRRSV MLV vaccination, such as circulation, evolution and reversion of MLV in the field. For the diverse strains of PRRSV at pig farm level, how can we do? How can we lessen the recombination frequency between MLV and field viruses? In China, the right ways are: (i) to consolidate internal and external biosecurity level of pig farms, prohibiting the introduction of any new PRRSV strain into farms and helping reduce/block the spread and circulation of PRRSV among pig herds; (ii) to minimize reasonably the use of MLV vaccines; (iii) to push forward the elimination of PRRSV on breeding pig farms and boar studs, constructing more PRRSV-free breeding herds/farms.

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U.S. experience with Seneca Valley virus-associated vesicular disease

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Introduction

The term 'idiopathic vesicular disease' (IVD) has been used to describe sporadic swine cases in which affected pigs display vesicles, pustules, ulcers, and/or erosions on snout, lips, oral cavity, tongue, skin, coronary bands, and interdigit with an unknown cause (4). The occurrence of swine IVD has been reported in the United States and other countries including Australia, New Zealand and Italy since early 1980's (21). All these cases tested negative for well-known vesicular disease pathogens of pigs, such as foot-and-mouth disease virus (FMDV), swine vesicular disease virus (SVDV), vesicular exanthema of swine virus (VESV) and vesicular stomatitis virus (VSV). In 2008 and 2012, case reports of swine IVD in which Seneca Valley virus (SVV) was detected, but not any of the vesicular disease pathogens mentioned above, were made from the United States and Canada (17, 23), suggesting that SVV may be a causative agent for vesicular disease.

Seneca Valley virus

It is a small non-enveloped RNA virus, and taxonomically belongs to and is the only member of the genus *Senecavirus* in the family *Picornaviridae*. In the literature, SVV and senecavirus A (SVA) have been interchangeably used as the virus name. According to the International Committee on Taxonomy of Viruses, "Seneca Valley virus" should be the virus name to be used, as *Senecavirus A* is the species name for the virus.

The origin of SVV is unknown. Seneca Valley virus was first discovered in 2002 as a cell culture contaminant, named SVV-001, at a human biomedical research center in the US East Coast region, which was speculated to be due to use of animal products, such as bovine serum or trypsin of porcine origin, in cell culture (9). Yet, a retrospective study conducted later revealed that SVV had circulated in US swine populations as it was infrequently detected in archived clinical specimens dating back to 1998 (12). Interestingly, SVV has taken a great attention in human medicine because of its oncolytic property since its discovery (9, 18, 19), whereas the virus was not considered significant in the US swine industry until after SVV was detected in pigs with IVD.

SVV-associated disease

During late 2014 and early 2015, several states in Brazil experienced outbreaks of vesicular disease manifested by cutaneous lesions on the snouts and coronary bands of sows and growing pigs. In addition, increased neonatal mortality was observed for a short period in many breeding farms (14, 15, 25). Some herds also experienced an increased incidence of neonatal scouring. Samples taken from clinically affected pigs tested negative for FMDV, SVDV, VESV and VSV; however, SVV was detected by PCR in vesicular fluid, serum and tissues (13, 14, 15, 25). Starting in summer of 2015, a rise in cases of IVD, which clinically resembled the cases in Brazil, among pigs at exhibitions, packing plants and commercial farms was noted in the United States (8, 10, 26, 28), alarming state and federal regulatory agencies and swine industry professionals due to the concern of foreign animal disease (FAD) or new emerging disease. Besides cutaneous lesions (hyperemia, vesicles, ulceration), affected animals showed acute lameness, lethargy and transient fever (8, 28). In some breeding herds, increased neonatal mortality was also observed (5, 7). All of the cases tested positive for SVV but negative for FAD agents to the US. No other microbial agents were consistently identified among the cases. Since the US incidence, vesicular disease cases testing positive for SVV have also been reported in China, Columbia and Thailand (20, 24, 27, 29).

Multiple groups in the US have been successful in reproducing vesicular disease after experimental inoculation with contemporary US SVV isolates via oro-nasal routes in 3-week-old pigs (6), 9-week-old pigs (16), finishing pigs (11), and

mature gilts (3). Under the experimental conditions given in each study, the inoculated pigs develop vesicular lesions on snout, coronary band, and interdigit between 2- and 7-days after inoculation, leading to coalescing erosion on the coronary band and snout that heals in 2-3 weeks. Older animals become lame due to damages in feet. The inoculated pigs are viremic for 5-10 days and shed the virus in feces and oral fluids for up to 3 weeks. Among organs examined, the virus preferably replicates to the highest titer and stays for the longest time (4-6 weeks post inoculation) in tonsil, which is in agreement with field observations. In contrast to the vesicular disease, the neonatal mortality syndrome has not been reproduced yet by inoculating SVV to pregnant gilts and sows or newborn piglets although transplacental infection of fetuses by the virus occurs (3).

Epidemiology and ecology of SVV and its associated disease

Pigs are a natural host of SVV and reservoir animals have not been identified. Feral swine may be susceptible to SVV as the virus has been isolated from it according to a record in GenBank® (https://www.ncbi.nlm.nih.gov/nuccore/EU271758). The presence of neutralizing antibody against SVV-001in a relatively high percentage of serum samples collected from non-diseased cows was reported, suggesting that cattle and possibly other farm animals can be exposed to SVV and could serve as a natural host (12). Yet, no further studies have been made to determine the susceptibility of feral swine and cattle and their role in maintaining and transmitting the virus.

Besides pigs and cattle, the presence of anti-SVV neutralizing antibodies at a low level (≤1:4) has also been demonstrated in sera collected from non-diseased mice (12). Diagnostic investigations on three swine farms undergoing IVD in 2015 has demonstrated the presence of SVV in not only swine specimens (lesion swabs, serum, tissues) but also feces and intestines from mice caught on the farms (11). Unexpectedly, some of internal (oral and rectal) or external (feet and fur) swabs which were collected from wild mice captured in Iowa's wetlands in 2015 for an influenza virus surveillance study were also positive for SVV RNA (Yoon K-J, unpublished data). These observations suggest that mice may play a role in transmission of the virus within farm or between farms in a close proximity.

The incidence rate of SVV-associated vesicular disease in the field appears to be higher during warmer weather seasons than cold weather seasons; however, the presence of SVV in pig populations is year around according to diagnostic laboratory data and no insect vector has been identified. Interestingly, detection of SVV RNA in flies caught inside and outside of buildings with pigs undergoing SVV-associated disease was reported (11), suggesting that flies could be at least a mechanical vector contributing SVV spread between rooms within a production facility once the virus is introduced.

It is unknown how prevalent SVV is in US swine population. In 2015 when increased SVV-associated IVD cases were noted, a surveillance study was conducted to examine oral fluids submitted to veterinary diagnostic laboratories in Iowa and Minnesota between August 24 and September 1 for SVV. In total, 2033 oral fluids from 441 swine operations without clinical signs of vesicular disease in 25 states were tested by PCR. Approximately 1.2% of the oral fluids representing 5 swine herds in 5 states tested positive for SVV RNA (https://www.aasv.org/news/story.php?id=8361), showing a relatively low prevalence of virus infection at the time when an increase of SVV-associated vesicular disease emerged in the US. Interestingly, Brazilian researchers have reported no serologic evidence of SVV circulation in Brazilian swine herds before 2014 when SVV-associated disease outbreaks started (22).

Knowing that SVV has been around in US swine for a long time, a sudden uptick of SVV-associated IVD in the US is still a mystery. One of logical assumptions would be a change in the virulence of virus which may have been associated with genetic changes since earlier attempts to reproduce the disease with 'old' strains of SVV failed. However, such an assumption could not be corroborated in a recent animal study at the USDA National Animal Disease Center as both older, including SVV-001, and 2015 US isolates of SVV were found to replicate in pigs comparably and cause vesicular disease under experimental conditions (2). Further studies remain to identify other factors contributing to increased incidences.

The economic impact of SVV-associated vesicular disease to individual farms and the US swine industry has not been estimated but is not expected to be high because: a) the virus infection causes no mortality except neonatal piglets; b) the

disease appears to be self-limiting; c) overall incidence rate is not high; and d) sows seem to develop the immunity and pass lactogenic immunity to newborns. Nonetheless, because vesicular disease associated with SVV infection is clinically similar to other well-known vesicular diseases such as FMD and SVD in pigs, there is the cost associated with diagnostics and business interruption particularly related to pig movement during diagnostic investigation by regulatory personnel. In breeding farms, neonatal mortality causes a productivity loss even for a short period.

Prevention and control

Biosecurity is an important prevention strategy for SVV like for many other swine pathogens. Replacement pigs and delivery or pick-up truck were identified as risk factors according to investigation on herds broke with SVV-associated vesicular disease (1). Therefore, an appropriate biosecurity protocol to address these risks, including appropriate testing plan for replacement pigs, should be in place. Feedstuffs should be viewed as a potential source of the virus depending upon storage condition and origin. Appropriate vermin control, particularly for rodents, should be implemented to reduce the risk of virus transmission. At this point, risk imposed by birds is unknown. When herds have an outbreak of SVV-associated vesicular disease, infected pigs shed the virus in feces which can be a source for environmental contamination including feeds. The virus is known to survive well in the environment and feedstuffs. Therefore, facilities and equipment should be cleaned and disinfected during and after outbreak. For disinfection, 5% house bleach has shown the effectiveness against SVV on all types of surface (https://www.swinehealth.org/results/). A commercial disinfectant like Synergize® is also effective but requires much longer time than 5% bleach.

As is the case for many swine diseases, effective prevention and control for SVV requires appropriate diagnostic testing and periodic monitoring. Pigs on a source farm for replacement animals should be tested for both the virus and antibody. Sera and oral fluids are samples of choice. For clinical animals, vesicular lesion swabs, vesicle fluids if available, sera, tonsil swabs should be obtained for virus testing (7). For serology, serum-virus neutralization assay and IgM-based serologic assays offer a merit for the early detection of virus-specific antibody as compared to indirect fluorescent antibody test (11).

There are no commercial vaccines for SVV available to veterinarians and producers. All strains of SVV known to date fall into the same serotype. The virus appears to induce good protective immunity against a subsequent challenge, which may last for 5-6 months after initial exposure and humoral immunity seems to be sufficient for disease protection (Kelly Lager, personal communication). Therefore, vaccination can be a viable option to consider for prevention and control of SVV if desired. An experimental inactivated whole virus vaccine has shown its efficacy against SVV-associated vesicular disease. However, the sporadic nature of SVV-associated vesicular disease and its relatively low overall economic impact might not justify the cost associated with vaccination at this point.

Conclusion remarks

The emergence of SVV-associated vesicular disease at a high rate alarmed the US swine industry, reminding the vulnerability of swine populations for emerging infectious diseases. On a bright note, this provided the opportunity to gain a better understanding of the pathogenesis, ecology and immunobiology of this lesser-known disease, not to mention the development of virus-specific diagnostics, which can assist veterinarians and pork producers to develop better control and prevention strategies for SVV and SVV-associated disease. Last but foremost, as viral vesicular diseases can be clinically indistinguishable from each other, the swine industry must remain diligent for any signs of vesicular disease and report them to authorities for appropriate investigation. Seneca Valley virus should be included in the differential list.

Keyword: swine vesicular disease, Seneca Valley virus, Senecavirus A, USA

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Swine foot-and-mouth disease--the key strategy for FMD control in China

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Foot-and-mouth disease (FMD) is one of the world's most economically important viral infectious diseases affecting cloven-hoof animals including cattle, pig, sheep, goats and more than 70 other wild species. While Europe, North American and Oceania maintain virus free status without vaccination, FMD is endemic in large areas of Indian sub-continent, South-east Asia, Central Asia, the Middle East, and Africa. In South Korea, Russia, Japan and some parts of South America, such as Paraguay, Ecuador and Venezuela, FMD has been reported sporadically. The etiological agent, FMD virus, belonging to *Aphthovirus* within the family *Picornaviridae,* exists in seven distinct serotypes, such as O, A, C, Asia1, South African Territories (SAT) 1, 2, and 3. There is no cross-protection between these serotypes. Serotypes O, A and C have had a worldwide distribution, with serotype O being responsible for the majority of the outbreaks globally. There are different topotypes or strains within each serotype, the different topotypes within each serotype are usually restricted to distinct geographical regions. Thus, FMDV topotypes within each serotype have been sub-divided into seven regional pools throughout the world. Huge outbreak occurs when trans-pool transmission of different strains take place.

FMD control in endemic areas is implemented by diagnostics, surveillance and regular mass vaccination. In FMD-free countries, control policies have been based on depopulation of infected and in-contact animals, together with restrictions on movement of animals and their products. However, a large proportion of experimental studies investigating FMDV pathogenesis and vaccinology have been performed in cattle during the long history of FMD control and eradication. Furthermore, in many regions, it is common practice to vaccinate only cattle, but not pigs, based on the assumption that this practice may be sufficient to prevent dissemination of a potential outbreak. In 1997 Taiwan, China, a FMD outbreak caused by a serotype O virus in pigs, the virus, known as Cathay topotype of serotype O, is highly adapted to and virulent in pigs, but attenuated in cattle. After the Taiwan, China, outbreak, swine FMD and its role in FMD epidemics has attracted the attention of scientists and field veterinarian. In order to effectively control and eradication FMD, it is important to understand the difference between pigs and cattle, especially in Asian countries, where half of the pigs in the world are raised in different breeding modes. The major features specific to swine FMD should be carefully considered.

The clinic severity and transmission capacity in pigs vary greatly depending on virus strains. The acute clinical FMD are usually more severe in pigs compared to ruminant species due to high density rearing. The serotype O Cathay topotype virus that caused an outbreak in Taiwan, China, in 1997, is more popular in Asian countries' pig herds and significantly attenuated or becomes clinically non-infectious in cattle. A serotype O PanAsia lineage virus, the causative agent of all of these outbreaks, is divided into PanAsia, PanAsia-2 and Ind2001 lineages. These strains are sensitive to cattle and cause severe clinical signs. They also infect pigs without constant circulation among pig herds. Another most frequently detected serotype O FMDV is South-east Asia (SEA) topotype, O/SEA/Mya-98 lineage strain. This virus is same in sensitivity and infection between pigs and cattle. The virus circulating between pig farms and cattle farms is the biggest challenge of FMD control in China. On the other hand, the serotype Asia1 virus caused FMD outbreaks in some areas of China since 2005. After more than 10 years of comprehensive immunization control, China has successfully extinguished the Asia 1 FMD, and has stopped immunization to strengthen quarantine monitoring and surveillance from this year. Although serotype Asia1 FMDV could infect pigs, the virus has weak transmission capacities in pigs, and it does not form a continuously circulation among the pig herds. This is one of the key factors for successful control of Asia 1 FMD in China.

The oral route is likely to be the most common portal of entry for pigs, although respiratory tract is the main route of FMDV infection as we all know. The pig requires as much as 600 times more than the exposure to aerosol virus required by a ruminant animal to cause infection. More swine FMD outbreaks are caused by contact with FMDV contaminated materials or equipment, especially feeding with untreated swill. The virus via the oral route gain entry through the

mucosal surface of the tonsils rather than lower gastrointestinal tract, then via viremia spread to epithelium. Persistent infection is a very important issue in FMD control. Pigs are more efficient in complete clearance of the infection, the live virus or viral ribonucleic acid is not detected from infected pigs after 28 days of infection, so the pigs do not become carriers. As we know, in FMDV infections, cattle are the indicators, goat and sheep are the reservoirs and pigs are the amplifiers. Pigs may exhaust vast quantities of airborne virus though their breathing, about 3000 times as much as cattle. In the UK in 2001, pigs played a very important role in the PanAsia lineage type O FMD outbreak, as the outbreak was original reported in pigs.

Vaccination is one of the comprehensive measures of FMD control. However, the immune response is relatively lower in pigs than in other species. Previously reported, vaccinating pigs require 4-10 times a dose of bovine water adjuvant vaccine, which means a high payload of effective antigen is required to elicit a protective response. In addition, the onset of immunity is usually 3-4 days in cattle and sheep, but these periods are longer in pigs, averaging 14 days after vaccination. Moreover, some researchers postulate that maternally derived antibodies (MDAs) do not always interfere with the development of inactivated vaccine induced immunity, but MDAs is obviously effective in the development of protective immunity response against FMD in pigs. The optimal vaccine program is particularly important for the prevention and control of swine FMD.

China is a large country in animal husbandry and consumer of pork. The pig industry plays an important role in stabilizing the supply of food and the income of farmers. By the end of 2016, the country has a total susceptible livestock population of 2.19 billion, of which 480 million are cattle, 590 million are sheep and goat, and 1.12 billion are pigs, almost half of the pigs in the world. In the strategy for controlling and eradicating FMD, the key is successful control of swine FMD in China. In the past, the outbreak of FMD was closely related to trans-boundary movements of animals and their products. In 1950s, serotype O and A FMD endemic in mainland China mainly were introduced from the north border. Serotype Asia 1 FMD was limited to the border areas between China and South-east Asia. In recent years, serotype O, A and Asia1 FMD are mostly epidemic in China. The epidemiological analysis show that viruses, such as O/SEA/Mya98, O/ME-SA/Pan-Asia, O/ME-SA/2001, Cathay strains, A/Asia/Sea-97 and serotype Asia1 Group-V strains are introduced from the south and south-east border areas, especially from illegal animal and products trade. Serotype Asia1 FMD has been successfully controlled and the nation-wide vaccination will stop in 2018. The Chinese government has adopted compulsory immunization measures through a large amount of manpower and financial resources to prevent and control FMD. The susceptible animals are immunized twice in spring and autumn respectively, the monitoring of immune antibody and the clinical etiology are strengthened every year. More and more FMD prevention and control experience indicates that when the virus adapt and continuously circulate in pig herds, swine FMD will be more difficult to control and eradicate. The role of pigs in the recent FMD epidemics has been significant, mainly in countries from Eastern Asia. Therefore we should make up for gaps in current scientific knowledge of ruminant species and swine FMD control to develop effective control and eradication measures for high density pigs area.

Antiviral strategies against PRRSV infection

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PRRSV is a major economically significant pathogen that has adversely impacted the global swine industry for almost 30 years. Since the identification and characterization of PRRSV, new virulent PRRSV strains have constantly evolved and caused new outbreaks around the world. To prevent PRRSV infection, multiple MLV vaccines against both genotypes have been developed. However, the prevalence of PRRSV infection in swine herds is still high and vaccination has achieved little success. The dilemma of PRRSV control has been somewhat surprising, since a number of vaccines against equine arteritis virus, another member of *Arterivirus*, are available and effective. Despite the sustained effort, PRRSV specific treatment for infected herds or prevention methods other than vaccines is still unavailable. Moreover, except for the highly mortality and morbidity, PRRSV infection leads hosts more susceptible to secondary infection of other viruses or bacteria. In the field practice, various management procedures have been implemented to achieve farm and regional control of PRRSV infection, which include PRRSV test for semen and gilt acclimation, removal of seropositive animal, herd depopulation and repopulation, and herd closure and rollover; however, control and elimination of PRRSV within a relatively large region is much more complicated and expected to require a much longer-term commitment. Therefore, effective PRRSV control and prevention methods are urgently needed. Here, we systematically describe recent advances in anti-PRRSV research, especially focusing on those techniques with the potential to transform current anti-PRRSV strategies. Furthermore, combination of these new techniques may provide creative insights to guide future PRRSV control and prevention.

Key words: PRRSV, antiviral, receptor-blocker, miRNA, natural products

Contemporary issues in the surveillance of pig diseases

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INTRODUCTION

Paskins (1999) defines surveillance as, "*activities aimed at ascertaining the health status of a given population with the aim of early detection ….*" and monitoring as "*all activities aimed at detecting changes in the epidemiological parameters of a specified disease*". By this definition, surveillance deals with disease detection and monitoring follows changes over time. For simplicity, we will use "surveillance" and "monitoring" as synonyms in this discussion.

Why talk about surveillance? We work on behalf of pig health, pig welfare, and producer economic well-being. The three main tools at our disposal are: **Biosecurity** to protect farms or regions from the introduction of infectious agents. **Herd immunity** to control endemic infectious diseases. **Surveillance** to verify that the biosecurity and herd immunity measures we have implemented are actually functioning. Schwabe (1982) described this as creating baselines against which "*effects of intervention (control) efforts can be measured*".

MODERN PORK PRODUCTION

Globally, pork production is evolving from small populations of pigs on many farms to large populations on fewer farms. This has been made possible by improvements in housing, e.g., sophisticated, well-engineered facilities that reduce labor, increase worker efficiency, improve pig health, and allow for management of larger populations. Simultaneously, we have changed the way we organize and manage pig populations, e.g., multi-site production, all-in-all-out production by building or site, wean-to-finish barns. These changes have directly or indirectly generated a massive increase in the numbers of live animals and quantities of meat, pork by-products, and feedstuffs moved across political boundaries (Dee et al., 2018).

Overall, these changes represent a net gain to society: today's farmers are able to produce more high-quality pork with fewer resources. However, the same infrastructure and production characteristics that have led to improved efficiencies, i.e., large herds with high throughput and extensive pig movement between sites, has left the industry vulnerable to the rapid dissemination of infectious diseases. Thus, we have struggled to control ASFV, PEDV, PRRSV, as well as our historic nemeses, CSFV and FMDV. Today, just 32 of the 181 OIE-member countries are free of CSFV and just 66 are free of FMD without vaccination (Knight-Jones and Rushton, 2013; Longjam et al, 2011; OIE, 2017a; OIE, 2017b).

STRUCTURE OF THE INDUSTRY AND IMPACT ON DISEASE

Herd size is relevant to this discussion because of its impact on disease. Very simply, increasing the number of individuals in a population leads to an increase in the size of outbreaks, the frequency of outbreaks, and the difficulty of achieving disease control (herd immunity). The effect of population size on disease is well-documented in humans, especially for measles (Haggett, 2000), but recent research has shown that the concept holds for swine, as well. This explains why, influenza, PRRSV, salmonella, and other pathogens become endemic as herd size increases (reviewed by Pitzer et al., 2016). Of course, "herd size" is really just a proxy for other fundamental characteristics of pig farms that affect the behavior of pathogens:

Subpopulations. Animals on contemporary farms are spatially separated by age, production stage, and/or function, with little interaction between subpopulations. Physical segregation is a necessary part of managing animals in modern pork systems, but a consequence of physical separation is the creation of subpopulations that often differ markedly in disease status. Current surveillance protocols have not developed practical guidelines for allocation of samples across subpopulations within farm complexes, but it can be shown that even sample allocation across subpopulations, i.e., uniform spatial allocation, achieves the highest power of disease detection, regardless of test performance or variability in

disease distribution.

Population dynamics. Subpopulations change rapidly, but non-uniformly, in space (buildings) as they move through the production cycle. A finishing barn on a typical farm will experience ~250% annual population change as groups of animals are placed in the facility, grow, and move to market. Although more stable than growing pig populations, ~40% of females in sow herd populations are replaced annually. The rate of population change in swine populations is important because it destabilizes herd immunity and promotes pathogen endemicity.

Metapopulations. Metapopulations are populations separated by space, but connected epidemiologically by the movement of individuals between them. For example, in 2013, ~29 million live pigs were exported from Belgium, Denmark, France, Germany, Hungary, the Netherlands, Poland, Portugal, and Spain and >24 million live pigs were imported by the same countries (www.fao.org/faostat). In the U.S., health papers are required to move pigs across state lines. Thus, we know the number of pigs moved between states (not to abattoirs) rose from fewer than 5 million pigs in 1990 to > 52 million pigs in 2016 (USDA NASS).

These changes have affected pig production and health in unanticipated ways. On the farm, they enabled the development of multifactorial diseases that resist traditional methods of prevention and control, e.g., porcine respiratory disease complex (Opriessnig et al., 2011; Schwabe, 1982) and enabled the endemicity of others, with PRRSV serving as a prime example. That is, economic studies have uniformly shown that PRRSV imposes substantial losses on swine health and productivity despite decades of efforts to control its effects (Holtkamp et al., 2013; Nathues et al., 2017; Neumann et al., 2005; Nieuwenhuis et al., 2012; Zhang and Kono, 2012). At the region and international levels, the result has been the rapid movement of pathogens, e.g., PEDV and ASFV, over long distances (Dee et al., 2018; Sánchez-Vizcaíno et al. 2012).

SURVEILLANCE FOR DISEASE DETECTION

A variety of surveillance designs are in use: passive vs active; syndromic vs test-based; random vs risk-based, etc. Friends who work in these methodologies argue passionately on their behalf. Regardless of the surveillance method, the first "positive signal" mandates diagnostic testing to fully characterize the problem, determine a course of action, and guide the response in an efficient and timely fashion. Whether at the farm level or the national level, the requirement for testing creates two problems: 1) How to easily, rapidly, and cost-effectively collect samples for surveillance? 2) How to determine sample size and sampling location in a statistically valid manner?

Samples. The cost of collecting individual animal samples, e.g., serum, nasal swabs, is prohibitive for routine surveillance. Oral fluid samples are a viable alternative because they are easily collected and because they are more diagnostically sensitive than individual samples, i.e., a better surveillance sample (Olsen et al., 2013). Oral fluid samples are readily collected from pigs by suspending a length of rope in an accessible location. Ropes are hung shoulder-high to the pigs in a clean area of the pen for 20-30 minutes. Oral fluids are collected as the pigs chew on the rope. To extract the sample, the wet end of the rope is inserted into a clean plastic bag and manually squeezed or mechanically compressed.

Tests. In human medicine, oral fluids have been used in surveillance for several decades for a variety of pathogens, including HIV (Frerichs et al., 1994) and measles (Ohuma et al., 2009). As was the case in human diagnostics, the use of oral fluids in swine medicine has grown as nucleic acid-based assays and antibody assays have been specifically adapted to this specimen. Although continued improvements in diagnostics can be expected, most swine pathogens and/or antibodies against them have already been reported in oral fluids: *Actinobacillus pleuropneumoniae* (Gonzalez et al., 2017), *Erysipelothrix rhusiopathiae* (Gimenez-Lirola et al., 2013), ASFV (Giménez-Lirola et al., 2016), CSFV (Grau et al., 2015; Panyasing et al., 2018), FMDV (Grau et al., 2015; Senthilkumaran et al., 2017a), influenza A virus (Goodell et al., 2013), PCV2 (Prickett et al., 2008), PEDV (Bjustrom Kraft et al., 2016), PRRSV (Kittawornrat et al., 2010, 2012), swine vesicular disease (Senthilkumaran et al., 2017b), and others. In North American, ~1.5 million tests were performed on swine oral fluids between 2010 and 2016 (Bjustrom Kraft et al., 2018).

Sampling. Conventional statistical sampling was introduced into swine surveillance in the 1970s and widely used in the Aujeszky's disease virus (ADV) eradication programs initiated after DIVA vaccines and ELISAs became available in the

1980s (Anderson et al., 2008; Cannon and Roe, 1982). The conventional (hypergeometric) sampling approach is based on the assumption that the characteristic of interest, e.g. ADV, is independent and homogenously spatially distributed in the population (Cochran, 1977). This was/is a reasonable assumption in extensive pig production. In contrast, contemporary production systems consist of heterogeneous hierarchical units (sites, barns, pens) within which contagious diseases move from pig-to-pig and pen-to-pen. Disease moves spatially, not randomly. As a consequence, two pigs located close to each other, e.g., members of the same pen or neighboring pen, are more likely to share the same disease status than two pigs distant from each other; a phenomenon termed "positive spatial autocorrelation" (Rotolo et al., 2017, 2018).

Spatial sampling performs better than conventional statistical sampling in the presence of spatial autocorrelation. Although little used in veterinary surveillance, autocorrelation is a common property of targets distributed in geographic space and, therefore, spatial sampling is widely used in environmental and social sciences (Wang et al., 2013). Because sample collections considering inherent spatial (and temporal) factors, spatial sampling actually requires fewer samples than conventional sampling to reach accurate and precise estimates (Haining, 2003; Griffith, 2005; Wang et al., 2012).

Surveillance guidelines for oral fluids. Our research group has worked extensively to develop sample size formulas and guidelines for oral fluid samples collected from group-housed animals (Rotolo et al., 2017). This is an active area of research which will produce new findings in the future. The following recommendations are based on our work:

1. Sample at the barn level (or airspace)

Animals in commercial production systems are segregated by age and stage, with little mixing between them. The result is that barns on a site are often of different infection status. Sampling across multiple barns on a site is a powerful approach for detecting infection or proving that the population on the site is truly negative.

2. Number of samples

Collect 2 to 6 samples per barn (or airspace) (Rotolo et al., 2017). A larger sample size may provide a higher probability of detection, but may not be practical, affordable, or necessary to meet the objective. Let the purpose of sampling and the budget drive this decision. The number of pens in a barn is not a driver in sample size selection. If the barn is designed with many pens, samples will likely be collected from separate pens. If the barn is designed with few pens, more than one sample per pen could be collected.

3. Use spatial sampling

A key feature is a fixed spatial approach: space samples equally over the length of the barn. Thus, to collect samples, hang ropes equidistant to each other in pens on alternate sides of the center alleyway over the length of the barn. For example, if two samples are to be collected, hang each rope approximately one-third of the length of the barn from each end. Whatever the number of samples to be collected, adjust the distance between ropes so that they are evenly distributed over the space occupied by the pigs.

4. Frequency of sampling

Sampling/testing should be done at regular intervals (every 1 to 4 weeks), depending on the purpose of surveillance. This frequency will provide a clear picture of pathogen circulation OR provide strong assurance that the site is negative. If it is necessary to choose, fewer samples collected at frequent intervals is more valuable than many samples collected infrequently. Collect from the same pens each time - repeated sampling of the same pens over time also provides a logical picture of pathogen shedding (PCR-based testing) and/or immune responses (antibody-based testing).

CONCLUSIONS

The swine industry has changed in ways that profoundly affect infectious disease ecology. In particular, our highly productive industry has evolved in ways that leave us highly vulnerable to infectious diseases. To respond effectively, we need to be able to cheaply and easily collect infectious disease data in a longitudinal process of analysis-and-response. On the farm, integration of surveillance data with production data could provide the means to: (1) identify the circulation of specific pathogens; (2) quantify their effects on pig health and productivity; (3) target interventions to the correct pathogen and population; and (4) time the intervention for maximum effect. At the regional and national levels, aggregate samples,

such as oral fluids, will make area control programs more practical, affordable, and successful.

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Oral Abstracts

VIII-1-007

A novel recombinant porcine reproductive and respiratory syndrome virus with significant variation in cell adaption and pathogenicity

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Introduction
PRRSV is the most important economical agent of swine industry. In this study, a recombinant strain GD1404 was isolated. Complete genomic sequence and pathogenicity of the strain were analyzed in order to be helpful for preventing and controlling this disease.

Materials and Methods
The GD1404 was isolated by primary PAMs. The third passage in PAMs was used for further experiments. MEGA6.0, RDP4 and BioEdit softwares were used to analyze the genome. To determine the pathogenicity of GD1404, three groups of PRRSV negative piglets were inoculated with GD1404, BB0907 and PBS respectively. After challenge, the temperature of each pig was observed and pigs were bled periodically. Necropsy was scheduled on 14dpi. Sections of lung were collected.

Results
GD1404 isolate was nonviable in MARC-145 cells. It was an inter-subgenotype recombinant of strains QYYZ and JXA1. The C-terminus of the GP2 protein of strain GD1404 had an amino acid deletion. Also, the ORF5a protein had 51 codons, five more than most other HP-PRRSV strains. Phylogenetic analysis based on ORF5 gene sequences showed that strain GD1404 and five others isolated in China formed a new subgenotype represented by strain QYYZ. GD1404 and HP-PRRSV BB0907 strains caused similar rates of mortality and interstitial pneumonia. However, strain GD1404 infection resulted in lower viremia and viral loads in the lungs, as compared with strain BB0907.

Conclusion
The results of this study provide evidence of the circulation of type 2 PRRSV QYYZ-like strains in China with variations in cell adaption and pathogenic abilities.

Keywords: porcine reproductive, recombination, ORF5a, GP2, pathogenecity

VIII-1-011

PRRS virus lineages determined in Mexico with 128 sequences obtained during a 2006 – 2013 period

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Introduction
PRRS is known as the most damaging disease in the history of swine production. Worldwide, PRRS viruses showed genetic divergence which in turn originated 2 viral types known to date. Mexico is infected by type 2 viruses. By comparing sequences (ORF5), a phylogenetic classification has been established with 9 lineages spread around the globe, however most of the published data in America, corresponds to USA/Canada findings and only a small note mention includes the lineage 5 as present in Mexico. The objective of this study was to generate information related to lineages of PRRS virus present in Mexico by genetic analysis of isolates obtained during a 7-year period.

Materials and Methods
128 viruses isolated from the states of Jalisco, Sonora, Puebla and Veracruz were collected between 2006 and 2013 and the ORF5 gene were sequenced and compared to sequences reported in GenBank. Finally, a dendogram was established using all sequences.

Results
We found the presence of lineages five lineages: L1, L2, L5, L8 and L9 in the country. Jalisco showed evidence of lineages L1, L5, L8 and L9. Sonora L1, L2, L5, L8 and L9, Puebla L1, L5 and L8. Veracruz L5. By using this new information and the date of the isolation, we made a frequency chart: L1 had 28% annual average frequency, L2 5.5%, L5 35%, L8 30% and L9 only 1.5%. Based on these data we could state that: L1 is becoming more frequent since 2007; L5 frequency decreased in 2006 – 2010 and then slowly and irregularly increased up to 2013, a situation probably related to the use of vaccines also belonging to L5. L8 remained constant; L9 was detected only in Jalisco and Sonora.

Conclusion
After 128 ORF5 sequences were analyzed, it was found that L5 is the most frequent lineage found in Mexico (35%), followed by L8 (30%), L1 (28%), L2 (5.5%) and L9 (1.5%).

Keywords: PRRS, lineages, Mexico

VIII-1-014

Genetic diversity of porcine reproductive and respiratory syndrome virus (PRRSV) isolates in the Netherlands from 2014-2016

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Introduction
Porcine reproductive and respiratory syndrome (PRRS) is the most significant swine disease worldwide and is endemic in many countries, including the Netherlands. It is caused by the PRRS virus which shows remarkable genetic variation. Epidemiological and molecular analysis of circulating PRRS-viruses is essential to review current diagnostic tools and key to modern farm management. Since the early nineties no epidemiological or molecular study has been performed on circulating PRRS-viruses in the Netherlands. To determine the genetic diversity of PRRS isolates in the Netherlands, the sequences of circulating field viruses were compared.

Materials and Methods
Seventy-six PRRS-virus isolates collected in the Netherlands from 2014-2016 were sequenced from and including ORF2 to ORF7. Phylogenetic analysis was performed using the MEGA 6.06 software and sequences were compared with sequences available on GenBank.

Results
All investigated isolates belong to the European type I viruses including twelve Lelystad-like viruses. Most isolates showed only approximately 90% similarity with published sequences in GenBank and suggest a Dutch cluster in the phylogenetic tree. Sequence comparison of the individual ORFs of the viruses with the Lelystad strain showed that ORF3 (84-89%) and ORF5 (82-89%) are the most diverse ORFs, whereas ORF2 (90-95%), ORF6 (88-97%)and ORF7 (89-97%) showed the highest similarity with the Lelystad strain. Furthermore, comparison of individual ORFs suggested that some of the isolates may have originated from strains with a different genetic background.

Conclusion
PRRS viruses isolated in the Netherlands in 2014-2016 show a high variation in their sequences. Most isolates belong to a distinct phylogenetic (Dutch) cluster within the type I viruses. The finding that some isolates have different origins when looking at the different ORFs suggests that recombination between different PRRS viruses may occur.

Keywords: PRRSV, epidemiology, sequence analysis, Netherlands

Ⅷ-1-016

Prevalence and recombination analysis of porcine reproductive and respiratory syndrome virus in recent years in China

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Introduction
Porcine reproductive and respiratory syndrome virus (PRRSV) is notorious for extensive genetic variation with recombination as one of the important mechanisms for its evolution. The current widespread of NADC30-like strains has caused much attention in China, especially concerning the frequent reports of the recombinant events between NADC30-like and other PRRSV strains. The objective of the present study was to analyze the prevalence, molecular variation and evolution of PRRSV in mainland China.

Materials and Methods
All available complete genome sequences of PRRSV from 2012 to 2017 in mainland China were downloaded from GenBank, including 272 strains. Genome sequence analysis was performed by using the DNASTAR package, DNAMAN 6.0 and the MEGA 6 software. Similarity comparisons were performed with SimPlot v3.5.1 within a 500-bp window sliding along the genome alignment (10-bp step size), the sequence to be analyzed was chosen as query sequence.

Results
HP-PRRSV-like viruses (173 of 272) remain predominant in mainland China. Meanwhile the isolation rate of HP-PRRSV MLV-derived strains (40 of 272) and NADC30-like strains (9 of 272) has been kept on a steady increase. Out of 272 strains, 38 were identified as the recombinant viruses, of which 30 strains were from the recombination of NADC30-like with other strains, indicating the highly recombinogenic nature of NADC30-like virus. A total of 108 breakpoints were detected from the 38 recombinant strains. Nsp2 displayed the most intricate recombination pattern with 36 breakpoints, and nsp9 is another breakpoint-rich region. Among structural proteins, ORF2 and ORF4 showed relatively complicated patterns of recombination.

Conclusion
The NADC30-like viruses have spread widely in mainland China, and display a highly recombinogenic characterization with intricate recombination patterns within nsp2. The widespread epidemic of NADC30-like strains and massive use of MVL vaccines badly increase the complexity and diversity of the domestic strains that have exacerbated the rate of PRRSV evolution in China in the most recent years.

Keywords: PRRSV, NADC30-like, recombination, breakpoint

VIII-2-009

Assessment of vertical transmission events in PRRSV1 endemic farms and dynamics of infection in maternities and nurseries

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Introducton
The objectives of this study were to determine the frequency of vertical transmission events of PRRSV1 in unstable farms and to assess the transmission of PRRSV within maternities and nurseries with regards to the frequency of vertical transmission events.

Materials and Methods
For the first objective, 387 animals of 11 farms suspected to be unstable for PRRSV were sampled at birth by taking umbilical cords (UC). For the second objective, two batches of 60 and 74 piglets (from 11 and 9 sows, respectively) born 5 months apart were sampled at day 1 (UC) and at 2, 3/4, 6/7 and 9 weeks of age (serum). RNA was extracted and the presence of PRRSV1 was determined by RT-qPCR.

Results
Overall, 24.8% of the UC tested were positive (accounting for 7/11 farms). In the first follow-up study, PRRSV positive UC were found in 12 piglets from 5 sows. In the second batch, 5 sows had at least one positive piglet at birth as well. In both batches, all animals but one which UC yielded Ct<35 were still viremic the 2nd week of age, while animals with UC yielding Ct>35 were negative at that time.

Conclusion
Therefore, contamination of UC during delivery probably overestimated the proportion of viremic-born piglets, but was a sensitive method to detect sows with vertical transmission events. Most of the piglets born infected (Ct<35) were still alive by 3-weeks of age (6/9; 66.7%) and by day 63, 66.7% survived. In the first batch, the percentage of viremic animals increased from 29.5% at 4-weeks of age to 89.5% at 63 days of age. In the second batch, viremic animals raised from 3.23% at 3-weeks of age to 27.87% at 6-weeks of age, with 10.34% of the animals infected at day 63. Most of the animals infected at birth had a high probability of survival throughout all the examined period, being thus a substantial source of infection for their penmates. Transmission within maternities was somewhat slower compared with the transmission in nurseries indicating that R value is probably substantially lower for animals with passive immunity.

Keywords: PRRSV1, unstable farms, transmission, umbilical cord

Ⅷ-2-017

PRRS virus detection at near-zero prevalence in neonatal piglets using processing fluid samples and applications for monitoring

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Introduction
To describe the collection of 'processing fluids' in commercial herds and to evaluate their use for monitoring PRRSV in low prevalence scenarios as compared with the tail swab sampling method.

Materials and Methods
'Processing fluid' (PF) is what we are calling the exudate from the tissues removed from piglets at the time of castration and tail docking. PF samples and 30 tail blood swabs ("matching sets") were collected on the same day and from the same piglet population in 5 breed-to-wean herds. The tail blood swab sampling technique was the PRRSV screening method used routinely in these herds. One of the herds was negative for PRRSV and was used as control. Each matching set consisted of one PF sample and 30 tail blood swab samples (tested in pools of five swabs). A total of 20 matched sets were collected from the five herds. Additionally, five PF samples were submitted for PRRSV (ORF5) sequencing.

Results
Farm staff were 100% successful in collecting processing fluids (PF). The number of pigs included in PF samples ranged from 250 to 710. The rate of PRRSV RNA detection by rRT-PCR in PF surpassed that of the matching 30 individual tail blood swabs (55% vs 10%). PRRSV-positive PF had cycle threshold (CT) values ranging from 30.5 to 35.1. PRRSV ORF-5 sequencing was successful in four of five samples submitted for that purpose. These four cases had a sequence homology greater than 99% with the wild type PRRSV identified in those herds with standard sampling methods.

Conclusion
Results of this study showed that this surveillance approach is easy to implement. In addition, paired tested from the same population showed that processing fluids were more likely to be PRRSV RT-PCR positive than tail blood swabs. Thus, processing fluids were a more effective method for monitoring PRRSV in suckling pigs than currently used methods. Farms undergoing virus elimination can use processing fluids to understand when PRRSV-negative pigs are being produced and accurately apply "MCREBEL" procedures, resulting in a greatly improved pre-weaning mortality during the elimination process.

Keywords: PRRSV, processing fluids, surveillance

VIII-2-020

Network analysis of swine shipments in China: the value for animal health, food safety and security

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Introduction
China's growing population, and increasing demand for animal protein, makes animal health and food safety and security more critical than ever. More than fifty-percent of the market demand falls on the pork industry. Understanding the pig movement patterns and trade network of China is crucial not only for early detection and rapid control of animal diseases but also to conduct risk assessments and disease spread and economic models to make more informed decisions in the pork value chain. This study is one of the very first to describe the spatio-temporal patterns and network characteristics of the pig trade network in a representative multi-site (>20 farms) facility in China.

Materials and Methods
We used social network analysis and space-time cluster analysis to describe the pig trade dynamics and identify locations and time periods that could be at highest risk for infectious disease introduction or spread using data of a big pig production system in China over a two-year period.

Conclusion
The resultant network mapping and spatial analyses will be use to illustrate the value of the use of pig movement networks for the control of diseases such as porcine reproductive and respiratory syndrome as well as epidemiological planning and emergency response of exotic or emerging diseases in China. Network description and properties will also provide the foundations for an accurate parameterization of infectious disease models of swine diseases, and the design of risk-based, more cost-effective surveillance, intervention, and risk mitigation strategies specifically adapted to the Chinese pork industry. Results presented in this study will help to minimize the economic and sanitary impacts of both endemic and emerging swine diseases and will contribute to the food safety/security and long-term sustainability of the China's pork industry.

Keywords: social network analysis, risk assessment, risk-based surveillance, disease modeling, geostatistical analysis

Ⅷ-2-026

Regional monitoring for PRRSV

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Introduction

PRRSV, an RNA virus is a fast evolving pathogen that continues to challenge the swine industry with new and potentially more virulent strains. As an example, in 2014 a virulent strain with an RFLP 1-7-4 pattern emerged in the US causing increased losses for the industry.

Materials and Methods

A pilot monitoring system was implemented in a US region on January 2017 with the objective of detecting newly emerging PRRS strains in a timely manner. Participants of the Morrison's Swine Health Monitoring Project (MSHMP), that collectively represent around 90% of the farms in the selected region, agreed to share historical and prospective PRRS ORF-5 sequence data. A total of 3,089 sequences were aligned using the Multiple Sequence Comparison by Log-Expectation (MUSCLE) algorithm and the similarity matrix was constructed. A PRRSV strain that had a similarity of <98% to any other sequence in the database was considered as new virus in the region that could have resulted from an introduction or recombination within the region. Results were communicated to participants monthly indicating whether new strains were detected, the farms and system affected, whether the detected new strains spread to other farms in the region, and the distance between farms affected with the new strain.

Results

During 2017, 492 PRRSV sequences were collected in the region. Only seven of them (1.4%) were considered as new viruses, while the rest (485) had at least one similar sequence detected in the region previously. Four of these seven new strains spread to other farms in the region during the year of monitoring, while the other three sequences did not spread. The distance between farms that shared these four newly detected strains was in most cases less than 8 miles. However, in one case a similar newly detected sequence was shared between farms 52 miles apart.

Conclusion

This pilot project provided timely information on new PRRS introductions to swine producers that may have helped them to take informed decisions regarding implementation of prevention and control strategies to limit the spread of new PRRSV strains in the region.

Keywords: PRRS, monitoring, regional

VIII-3-004

Peptide sequence domains in SRCR5 of CD163 that contribute to recognition by PRRSV-2

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Introduction
CD163 is a receptor for porcine reproductive and respiratory syndrome virus (PRRSV). CD163 is found on macrophages belong to M2. The extracellular region of CD163 possesses nine scavenger receptor cysteine-rich (SRCR) and two proline-serine-threonine (PST) domains.

Materials and Methods
HEK293T (HEK) cells transfected with domain-deleted constructs fused to enhanced green fluorescent protein (EFGP) were infected with a PRRSV-2 isolate expressing a red fluorescent protein (RFP).

Results
The results showed that cells expressing deletions of the 101 amino acid SRCR5, the interdomain region between SRCR4 and 5, or the 16 amino acid PSTII domain did not support infection. The deletion of SRCR8 and 9 had a lesser effect on infection. Insertion of proline-arginine (PR) dipeptides along the SRCR5 polypeptide was used to probe secondary and tertiary structures involved in infection. The PR insertions were created by inserting *Sac*II sites (CCGCGG) along the SRCR5 cDNA region. Four PR insertions possessing the greatest effect on infection were identified at positions 9, 48, 55 and 100. Growth curves and limited dilution titration studies confirmed the negative impact of the PR insertions. Computer modeling showed that the regions interrupted by the PR insertions comprise a well-defined binding pocket consisting of antiparallel β4 and β7 strands along with two opposing loop structures, located between β1/β2 and β4/β5. Replacing individual cysteines with alanines as a means to disrupt disulfide bonds also had a negative effect on infection.
Conclusion: The results from this study identify likely contact regions and structural requirements in CD163 involved in forming the interaction(s) between the macrophage and the corresponding PRRSV protein(s).

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Keywords: PRRSV-2, CD163

Ⅷ-3-014

Interaction of PRRSV nsp1-beta and the cellular protein nucleoporin 62 inhibits host mRNA nuclear export and host cell gene expression

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) blocks host mRNA nuclear export to the cytoplasm and inhibits host protein translation to facilitate its own replication. Subsequently, PRRSV nsp1-beta has been identified as the responsible protein for this function. In this study, the nsp1-beta-mediated inhibitory mechanism was investigated.

Materials and Methods

By nuclear-cytoplasmic fractionations and RT-qPCR, it was apparent that the blocking of host mRNA nuclear export was universal regardless of mRNA species. However, inducible genes including type I interferons were affected the most by nsp1-beta when stimulated. By immunofluorescence and confocal microscopy, nsp1-beta was found to co-localize with nucleoporin 62 (Nup62), indicating their specific interaction. This interaction was confirmed by mammalian two-hybrid luciferase assay and GST-pull down assay. Nup62 is one of the major components of the nuclear pore complex (NPC) that form channels spanning the double lipid bilayer of the nuclear envelope for nucleocytoplasmic transport of cellular molecules including host mRNAs. A region representing the C-terminal 328-522 residues of Nup62 was further identified as the binding domain to nsp1-beta. The C-terminal domain of Nup62 interacts with nuclear transport receptors and anchors Nup62 to NPC, suggesting the interaction with nsp1-beta disrupts NPC structure. Mutational studies revealed that leucine 126 of nsp1-beta was the critical residue for Nup62 interaction. Nsp1-beta L126A did not bind to Nup62 and host mRNA nuclear export occurred normally.

Conclusion

Our data demonstrate that PRRSV nsp1-beta binds directly to the C-terminal region of Nup62, and leucine at 126 is essential for this interaction and to block host mRNA nuclear export.

Keywords: PRRSV, nsp1-beta, Nup62, nuclear pore complex, host gene expression

VIII-3-025

Network mapping reveals regulated interactions among PRRSV nonstructural proteins

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Introduction
Porcine reproductive and respiratory syndrome virus (PRRSV) is an important member of the family *Arteriviridae*. Synthesis of the viral RNA is directed by replication/transcription complexes (RTCs) that are mainly composed of a network of PRRSV nonstructural proteins (nsps). However, the molecular details of how PRRSV nsps come together to assemble the viral RTCs have remained poorly understood.

Materials and Methods
In this report, we mapped the protein-protein interactions among PRRSV nsps by yeast two hybrid, coimmunoprecipitation and cotransfection assays.

Results
Our studies established a comprehensive interaction map for PRRSV nsps and identified important players within the network. More importantly, we revealed several regulated interactions that involved core enzymes nsp9 and nsp10.

Conclusion
Together, our data indicate that there exists a complex, regulated interaction network among the PRRSV nsps. The findings suggest an orchestrated mechanism for assembly of PRRSV RTCs and provide a foundation for better understanding the biological functions of PRRSV nsps in the virus life cycle.

Keywords: PRRSV, regulation, interaction

Ⅷ-3-021

Identification of the RNA pseudoknot within the 3' end of PRRSV genome as pathogen associated molecular pattern to activate antiviral signaling via RIG-I and TLR3

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Introduction
Porcine reproductive and respiratory syndrome virus (PRRSV) is the causative agent of porcine reproductive and respiratory syndrome (PRRS), characterized by reproductive failure in sows and respiratory diseases in pigs of different ages. It often leads to persistent infection and inefficient vaccination. The sensing mechanism in the type I IFNs induction of PRRSV remains to be fully clarified.

Materials and Methods
Here, we demonstrated that PRRSV activated the production of type I IFNs, but not the type III, in PAMs, which was relevant to RIG-I and TLR3. Moreover, the pathogen associated molecular patterns (PAMPs) of PRRSV and the host associated pattern recognition receptors (PRRs) were identified. We clarified that the pseudoknot in the 3' UTR of PRRSV genome, which is functional in RNA synthesis and virus replication, directly bound to RIG-I and served as a PAMP to stimulate the production of type I IFNs. To define the contribution of 3' UTR pseudoknot, the interaction between the two terminal stem-loop structures of the pseudoknot was disrupted by nucleotide mutation without changing the predicted structure of the individual hairpins.

Results
The results showed that the pseudoknot variants exhibited much weaker function in the IFN response. Furthermore, the RNA transcripts of the 3' UTR pseudoknot suppressed PRRSV replication in PAMs. In addition, the similar structures of equine arteritis virus (EAV), lactate dehydrogenase-elevating virus (LDV) and simian hemorrhagic fever virus (SHFV) exhibited the same effect.

Conclusion
These findings identify the pseudoknot structure of PRRSV and the other members of arteriviruses serves as a PAMP for immune recognition, which activates antiviral signaling via interaction with RIG-I and TLR3. Our results will contribute to our understanding of PRRSV pathogenicity and the development of the antiviral strategies.

Keywords: PRRSV, PRRs, PAMPs, pseudoknot, IFN

VIII-3-017

Porcine reproductive and respiratory syndrome virus induces stress granules closely associated with viral replication complexes and suppression of host translation

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Introduction
Stress granules (SGs) are dynamic sites of cytosolic mRNA storage that are formed in response to stress conditions, including viral infection. SGs have been implicated in regulating several aspects of the host-immune response to various pathogens. Porcine reproductive and respiratory syndrome virus (PRRSV) is a single-stranded, positive-sense RNA virus in the family *Arteriviridae* that poses an immense economic threat to the global swine industry. PRRSV suppresses several facets of the host's immune response and is therefore a challenge for control via vaccination.

Materials and Methods
Here in this study, we found that PRRSV strain VR2385 induces the SG response, as demonstrated by the phosphorylation of eIF2α and concomitant arrest in cellular translation. Formation of *bona fide* SGs were also observed at late time points post-infection, as indicated by the recruitment of marker proteins G3BP1, G3BP2, TIAR and eIF3b to SGs. The PRRSV-induced SGs were observed in close proximity to viral replication complexes (VRCs). Additionally, treatment with a specific inhibitor against the eIF2α kinase PERK suppressed PRRSV-induced SG formation, but did not inhibit viral replication. Furthermore, impairment of SG assembly by the siRNA-mediated knockdown of G3BP1 and G3BP2 did not affect the virus replication ability.

Results
Collectively, our results suggest that PRRSV-induced SGs may be involved in, but are not required, for efficient viral replication. However, the role of SGs in regulating the immune response to PRRSV still warrants further investigation in the future.

Conclusions
Better understanding of the molecular events involved in regulating SG formation in PRRSV-infected cells will allow for rationale development of safer and more effective vaccines against PRRSV.

Keywords: PRRSV, stress granules, host translation

Ⅷ-3-030

Proteomic analysis of the secretome of porcine alveolar macrophages infected with porcine reproductive and respiratory syndrome virus

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Introduction
Porcine reproductive and respiratory syndrome virus (PRRSV) causes porcine reproductive and respiratory syndrome (PRRS), which is characterized by reproductive failure and respiratory disorders. In response to PRRSV infection, cells secrete a broad range of proteins that communicate to virus-infected and noninfected cells to trigger and boost the host immune and inflammatory responses. Viruses also exploit the secreted proteins to facilitate their replication and immune evasion. This study focused on the role of secretes proteins in virus infection by secretome analysis of porcine alveolar macrophages Infected with PRRSV.

Materials and Methods
The secretome of PRRSV-infected porcine alveolar macrophages (PAMs) was analyzed by label-free quantitative proteomics. Data resulting from the triplicated MS/MS experiments were processed using MaxQuant software (version 1.3.0.5). The proteins were blasted against the UniProt database. Differentially expressed proteins between PRRSV- and mock-infected cell supernatants were screened with the two-tailed Student t-test with a probability (*P*) value of <0.05 and a quantitative ratio of >1.5 or <0.667. Enrichment of the differentially expressed proteins was identified using the GO program Blast2GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis with the hypergeometric test.

Results
A total of 95 secreted proteins with differentially expressed levels between PRRSV- and mock-infected PAMs were screened. Among these, the expression levels of 49 and 46 proteins were up-regulated and down-regulated, respectively, in PRRSV-infected cell supernatants, as compared with mock-infected cell supernatants. Bioinformatic analysis revealed that the differentially expressed proteins were enriched in several signaling pathways related to the immune and inflammatory responses, such as the Toll-like receptor signaling pathway and NF-kappa B signaling pathway, and involved in a great diversity of biological processes, such as protein binding and localization, as well as immune effector processes. In addition, PRRSV-infected cell supernatants induced significant expression of inflammatory cytokines in vascular endothelial cells.

Conclusion
The secretomic data obtained from PRRSV-infected PAMs in this study provide a comprehensive overview of proteins secreted by PAMs in response to PRRSV infection and suggest that the secreted proteins played potential roles in the host immune and inflammatory responses as well as PRRSV replication, thereby providing new insights into cell-to-cell communication during PRRSV infection.

Keywords: porcine alveolar macrophages, porcine reproductive and respiratory syndrome virus, proteomics, secretome

Ⅷ-3-032

Nsp2 is crucial for highly pathogenic porcine productive and respiratory syndrome virus to trigger high fever in pigs

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is caused by PRRS virus (PRRSV). In 2006, Atypical PRRS caused by a highly pathogenic PRRSV variant (HP-PRRSV) broke out in China. The atypical PRRS is characterized by high fever (>41℃ for at least 4 days), high morbidity and high mortality in pigs of all ages. Prostaglandin E_2 (PGE2), derived from arachidonic acid (AA) through the activation of the limited enzymes cyclooxygenase type 1/2 (COX1/2), plays an important role in fever induction *in vivo*. Our previous work shows that HP-PRRSV infection increases the production of PGE2 in porcine alveolar macrophages (PAMs) via the activation of COX-1.

Results

Here, we further showed that HP-PRRSV infection up-regulated PGE2 production through the activation of COX-2 via MEK1-ERK1/2-C/EBPβ signaling pathways in microglia cells. And then, we investigated whether or which PRRSV structural protein or nonstructural protein could induce the activation of COX-2. Our results showed that PRRSV nonstructural protein 2 (NSP2) had the ability to up-regulate COX-2 production through activating MEK1-ERK1/2-C/EBPβ signaling pathways. Using the constructs of deletion mutants, we demonstrated that 499-566 aa and 628-747 aa of NSP2 were crucial for NSP2 to induce COX-2 up-regulation and PGE2 production. Finally, we constructed the recombinant HP-PRRSV with the deletion of 499-566 aa, 628-747 aa, or both regions in NSP2, and found that the recombinant HP-PRRSV had impaired ability to induce high levels of PEG2 production and fever.

Conclusion

These data may help us understand the molecular mechanisms underlying the high fever induced by HP-PRRSV and provide us some clues for the development of HP-PRRSV attenuated vaccines.

Keywords: HP-PRRSV, high fever, PEG2, COX2, nsp2

VIII-3-033

Identification of sites in GP2a of type 1 porcine reproductive and respiratory syndrome virus (PRRSV1) responsible for MARC-145 adaptation

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Introduction
Pulmonary alveolar macrophages (PAM) are known to be fully permissive to PRRSV infection and may support viral replication. They are currently the most extensively used cells to study PRRSV replication cycle. In addition, the MARC-145 cell line has been proven to be valuable for viral attenuation with regard to vaccine development and mass vaccine virus production. Cell-adaptation is necessary to grow the virus on MARC-145 cells. Through sequence alignment of multiple wild-type and MARC-145 cell-adapted PRRSV1 strains, consistent amino acid substitution in GP2a were observed in MARC-145 cell-adapted strains.

Materials and Methods
To investigate the putative effect of the substitutions at these positions, the aforementioned substitutions were introduced in the background of a full-length infectious cDNA clone of the PRRSV1.1 13V091 strain via site-directed mutagenesis. Introduction of a single amino acid substitution at either position A or B and a double substitution (A+B) was followed with the recovery of viable recombinant viruses denoted as Mut A, Mut B and Mut A+B, respectively. Subsequent sequencing of the recombinant viruses confirmed the presence of the introduced amino acids at the correct positions. Replication kinetics of these recombinant viruses was assessed in order to determine the effect of the introduced mutations on the viral replication. No differences could be observed when comparing the multi-step growth curves obtained in PAM. However, when comparing the replication kinetics in MARC-145 cells, a positive effect (+0.8 $\log_{10}$ compared to wild-type virus) on the growth characteristics of the 13V091 strain could be observed upon introduction of a single amino acid substitution at position A of the GP2a.

Results
Interestingly, when introducing a mutation at position B, no virus replication was observed on MARC-145 cells. However, the double mutant Mut A+B showed the highest viral replication compared to the other viruses (+2.1 $\log_{10}$), suggesting that the double mutation is a determining factor in PRRSV1 adaptation to MARC-145 cells. However, the molecular basis of the adaptation mechanism of PRRSV1 still needs to be determined.

Conclusion
The results obtained within this study allow an in silico MARC-145 cell adaptation of PRRSV1, allowing a favor development of novel vaccines.

Keywords: PRRSV, GP2a, MARC-145, adaptation, adaptation mutation

VIII-4-002

ORF1a of highly pathogenic PRRS attenuated vaccine virus plays a key role in neutralizing antibody induction in piglets and virus neutralization in vitro

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Introduction

Currently, porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most economically important viral pathogens in swine in most countries, especially China. Two PRRSV attenuated live vaccine strains (HuN4-F112 and CH-1R) are currently widely used in China. Our previous study showed that HuN4-F112, but not CH-1R, induced high anti-nucleocapsid (N) antibody and neutralizing antibody (NA) titers. Additionally, sera from HuN4-F112 inoculated pigs induced low cross neutralization of CH-1R.

Materials and Methods

In the present study, 6 chimeric viruses through exchanging 5' untranslated region (UTR)+open reading frame (ORF)1a, ORF1b, and ORF2-7+3'UTR between HuN4-F112 and CH-1R were constructed and rescued based on the infectious clones of rHuN4-F112 and rCH-1R. The characteristics of these viruses were investigated *in vitro* and *vivo*.

Results

The results indicated that all the three fragments, 5'UTR+ORF1a, ORF1b, and ORF2-7+3'UTR, could affect the replication efficiencies of rHuN4-F112 and rCH-1R *in vitro*. Additionally, both 5'UTR+ORF1a and ORF2-7+3'UTR affected the anti-N antibody and NA responses targeting rHuN4-F112 and rCH-1R in piglets. Based on these findings, we concluded that the 5'UTR+ORF1a region of HuN4-F112 played a key role in inducing NAs in piglets. Furthermore, we confirmed for the first time that ORF1a contains a neutralization region.

Conclusion

This study provides important information that can be used for further study of the generation of anti-PRRSV NAs.

Keywords: PRRSV, chimeric virus, neutralizing antibody, neutralization region

Ⅷ-4-005

PRRSV nucleocapsid protein binding to PIAS1 activates NF-κB for production of proinflammatory mediators

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Introduction

PRRSV triggers the onset of inflammation during infection, and proinflammatory cytokines including interleukin (IL)-1beta, IL-6, IL-8, and TNF-alpha have been shown to be upregulated in virus-infected porcine pulmonary alveolar macrophages (PAMs), suggesting the activation of NF-κB by PRRSV.

Results

We show that in cells expressing PRRSV nucleocapsid (N) protein, the RelA (p65) subunit of NF-κB became increasingly phosphorylated and translocated to the nucleus resulting in its activation. By yeast two hybrid screening using N as a bait, the protein inhibitor of activated STAT1 (PIAS1) was identified from PAMs as a molecular partner of N. PIAS1 binds to RelA and prevents NF-κB activation by interfering RelA-DNA binding in the nucleus and thus functions as a negative regulator of NF-κB. The N binding to PIAS1 was confirmed by coimmunoprecipitation and colocalization studies. To determine the binding domain in the PIAS1 and PRRSV N proteins, series of deletions and truncations were constructed. The binding domain of PIAS1 was mapped to the N-terminal fragment, and this domain was sufficient to bind to RelA and prevent its binding to the κB site, demonstrating the competitive binding between N-PIAS1 and NF-κB-PIAS1. For N, the region between 37 and 72 amino acid residues was found to interact with PIAS1, and this region overlapped the nuclear localization signal of N. This region was shown to activate the NF-κB signaling, confirming the correlation between N-PIAS1 binding and NF-κB activation. By competition assay, we show that the binding of PIAS1 to N is preferred to RelA, and this preferred binding was validated in PRRSV-infected cells.

Conclusion

In summary, PRRSV N binds PIAS1 and releases NF-κB from PIAS1, and as a consequence, NF-κB becomes activated. This is the novel mechanism of PRRSV for NF-κB activation.

Keywords: PRRSV, nucleocapsid, NF-κB, PIAS, inflammatory cytokin

VIII-4-010

The let-7 family of miRNAs inhibits replication of porcine reproductive and respiratory syndrome virus *in vitro* and *in vivo*

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Introduction
Porcine reproductive and respiratory syndrome (PRRS), which causes enormous economic losses to the worldwide swine industry, is a viral disease caused by PRRSV. An increasing number of studies have shown that miRNA plays a vital regulatory role in immune response and development of disease. In previous studies, we found the let-7 family of miRNAs was differentially expressed before and after PRRSV infection in different pig breeds.

Materials and Methods
ViTa was used to predict the potential target sites of let-7 family in PRRSV 3'UTR. The target genes of let-7 family was predicted by using the TargetScan. The luciferase assay, qPCR, western blot and IFA were used to detect the regulatory effect of let-7 family on IL6 and PRRSV. The role of IL6 in the replication process of virus was studied by siRNA technology. Co-expression plasmid pEGFP-N1-Let-7 of the let-7 family was constructed by fusion PCR, and then intramuscularly injected into piglets to detect the let-7 regulation on PRRSV in vivo.

Results
By bioinformatics prediction and experiment, the results demonstrate that PRRSV replication is regulated by the let-7 family, which binds to the PRRSV 3'UTR and also directly regulates the expression of the proinflammatory cytokine IL6. To understand the role of IL6 in viral replication, inhibition of IL6 expression in Marc-145 cells showed to facilitate the replication of PRRSV at both mRNA and protein levels. At the same time, co-expression plasmid pEGFP-N1-Let-7 of the let-7 family members could significantly decrease IL6 expression by intramuscular injection into piglets. Furthermore, PRRSV infected pigs treated with pEGFP-N1-Let-7 showed substantially decreased viral loads in PAM cell and relief from PRRSV-induced fever compared to negative control (NC).

Conclusion
Overall, the results show that the let-7 family inhibits PRRSV replication and IL6 expression both in vivo and in vitro. Proliferation of PRRSV causes the expression of a large amount of the proinflammatory cytokine IL6, leading to inflammatory reactions and fever. Therefore, Let-7 can regulate the PRRSV replication and reduce the inflammatory reaction by acting on PRRSV 3'UTR and host proinflammatory factor IL6. Our findings indicate that let-7 family can be used as antiviral therapy against PRRSV infection.

Keywords: let-7, PRRSV, IL6, regulation

VIII-4-013

Genetic programming of porcine memory B cells to enable the isolation of PRRSV-neutralising monoclonal antibodies

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Introduction

Broadly neutralising antibodies are the subject of intense research in the context of vaccine development for a number of highly variable viruses, including HIV and influenza. Central to these efforts are methods to generate and analyse the specificity of naturally occurring monoclonal antibodies (mAbs). The rapid evolution of PRRS viruses (PRRSV) poses a major challenge to effective disease control since available vaccines show variable efficacy against divergent strains. Knowledge of the antigenic targets of virus-neutralising antibodies that confer protection against heterologous PRRSV strains would be a catalyst for the development of next-generation vaccines. Key to discovering these epitopes is the isolation of neutralising mAbs from immune pigs.

Results

To address this important unmet need, we are evaluating an innovative approach which involves genetically programming memory B cells by transduction with a retroviral vector expressing the Bcl-6 transcription factor and the anti-apoptotic Bcl-xL. We have demonstrated that this technology efficiently converts porcine memory B cells into proliferating Ab-secreting cells, which retain their surface immunoglobulin expression. We have produced a cohort of hyperimmune pigs by experimental sequential challenge infection with heterologous PRRSV strains, which has resulted in high serum neutralising antibody titres against PRRSV-1 (mean ND_{50} 330) and lower titres against PRRSV-2 (mean ND_{50} 14).

Conclusion

The results of on-going screens to isolate PRRSV-neutralising mAbs will be presented and discussed. We hope that the isolation of these mAbs will contribute to both our understanding of the nAb response to PRRSV and allow epitopes to be resolved that may ultimately guide the design of immunogens to induce cross-protective immunity.

Keywords: PRRSV, B cell, monoclonal antibody, neutralisation

Ⅷ-5-013

Development of a broadly protective vaccine against porcine reproductive and respiratory syndrome virus using a synthetic strategy

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Introduction
Porcine reproductive and respiratory syndrome virus (PRRSV) continues to be one of the most important viral pathogens currently affecting the global pig industry. Current PRRSV vaccines are ineffective at providing adequate levels of protection against heterologous PRRSV strains, presumably because PRRSV strains circulating in the field are genetically divergent from the vaccine strains.

Materials and Methods
To design more effective PRRSV vaccines by overcoming the genetic divergence challenge, we recently generated a synthetic PRRSV strain containing a consensus genomic sequence (designated PRRSV-CON) based on a set of 59 non-redundant full-genome sequences of PRRSV-2.

Results
Through the immunization/challenge experiments in a young pig model, we demonstrated that this synthetic PRRSV-CON confers optimal levels of heterologous protection. PRRSV-CON at passage 1 is highly virulent and therefore, is not suitable to be used as a vaccine in pigs. We then successfully attenuated PRRSV-CON by continuously passaging the virus in a non-natural host cell line, MARC-145 cells. The attenuated PRRSV-CON confers similar levels of heterologous protection as its parental strain, indicating that attenuated PRRSV-CON can be an excellent candidate for PRRSV modified-live virus vaccines. Moreover, we found that PRRSV-CON has a unique phenotype of inducing type I IFNs instead of suppressing these cytokines like most of the wide type PRRSV strains do. The gain- and loss-of-function studies on MARC-145 cells revealed that the 3.3 kb genomic region of PRRSV-CON encoding viral proteins nsp1α, nsp1β and the N-terminal part of nsp2 was correlated to its IFN-inducing phenotype. The virus' capability of type I IFN induction may contribute to protective immunity against heterologous PRRSV strain.

Conclusion
Taken together, the results provide useful and novel information for designing more effective PRRSV vaccines with improved levels of protection against heterologous PRRSV strains.

Keywords: PRRSV, synthetic virus, innate immunity, heterologous protect, modified-live vaccine

Ⅷ-5-026

Efficacy of Ingelvac PRRS® MLV against a heterologous PRRSV 1-3-4 RFLP challenge

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Introduction
The objective of this study was to evaluate the efficacy of two commercially available PRRSV vaccines in a three-week-old pig respiratory challenge model, using a heterologous PRRSV RFLP 1-3-4 field strain, isolated in 2016.

Materials and Methods
At approx. three weeks of age (D0), 102 PRRSV naïve piglets pigs were randomized into groups, and intramuscularly vaccinated with 2 mL of either a placebo (n=36), Ingelvac PRRS® MLV (n=33) or Fostera® PRRS (n=33). Pigs were housed by group during the vaccination period. At D28, all pigs were comingled and challenged with 1 mL intramuscularly and 2 mL intranasally of diluted serum containing 7.97 log gc/mL of PRRSV 1-3-4. Serum samples were collected periodically from D0 through the end of the study on D42. Serum samples were tested by qPCR for viremia and by ELISA for anti-PRRSV antibody. Viremia testing for PRRSV was done by BIAH-Health Management Center, Ames, IA using qPCR. Results were reported as positive/negative, in addition to PRRSV quantity in units of genomic copy number per mL serum. Pigs were weighed at D0, day of challenge (D28), and termination of the study (D42) to assess average daily gain (ADG). On D42, all pigs were necropsied and lungs were scored for macroscopic lesions.

Results
The challenge isolate selected resulted in severely impacted ADG (-0.25 lbs), severe lung lesions (58.3%) and mortality (61.1%) in the challenge control group.

Conclusion
The results confirmed anecdotal evidence of the severity of disease associated with this PRRSV 1-3-4 isolate. Despite this virulent challenge, Ingelvac PRRS® MLV demonstrated heterologous protection by significantly reducing lung lesions, post-challenge viremia and mortality, while improving ADG compared to the challenge controls. Pigs vaccinated with Ingelvac PRRS® MLV had significantly higher ADG compared to the Fostera® PRRS vaccinated group. This study is another example demonstrating the ability of Ingelvac PRRS® MLV to protect against a relevant and contemporary PRRS challenge.

Keywords: PRRSV, heterologous protect, vaccine

VIII-5-033

Vaccination of 1-day-old pigs with a new porcine reproductive and respiratory syndrome (PRRS) modified live attenuated vaccine confers 26 weeks duration of immunity (DOI)

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Introduction
Porcine reproductive and respiratory syndrome (PRRS) is a major disease impacting the swine industry, that is often complicated with co-infections and resulting in important economic losses. The respiratory form of PRRS impact growing-finishing pigs, and effective protection against the virus is a major goal for veterinarians and producers. Suvaxyn® PRRS MLV is the first vaccine licensed in Europe for use in pigs 1 day of age. The vaccine is intended to be used in a single dose vaccination schedule, thus it is crucial to ensure protection of the pigs from vaccination to slaughter. The objective of the study was to evaluate the Duration of Immunity (DOI) of Suvaxyn PRRS MLV in pigs vaccinated at 1 day of age by intramuscular route, upon challenge with a PRRS-1 isolate as a respiratory challenge at 26 weeks of age (post-vaccination).

Materials and methods
Thirty-eight 1-day-old piglets, born from PRRSV seronegative sows, were divided into two groups. One group (20 pigs) was kept as negative control, and the other group (18 pigs) was vaccinated at 1 day of age with Suvaxyn PRRS MLV. The animals were challenged by IN route with Olot/91 strain 26 weeks after vaccination. Viral load in serum, rectal temperatures, shedding, clinical signs and body weights were evaluated. Nine to ten days after challenge pigs were necropsied and lungs scored for macroscopic lesions.

Results
The vaccine showed protection by a significant reduction of viral load in serum (3.8 log reduction) and in nasal shedding (1.0 log reduction) in comparison with negative controls. The vaccinated pigs also showed a reduction in macroscopic lung lesions, 3.7% versus 1.0% in controls and vaccinated respectively.

Conclusion
Vaccination with a single administration of the Suvaxyn PRRS MLV to 1 day-old seronegative pigs by IM route conferred a duration of immunity of 26 weeks, as seen by the significant reduction on the viral load detected in serum after challenge 26 weeks post-vaccination. Efficacy was also supported by the significant reduction on the percentage of lung lesions at necropsy, as well as the reduction of nasal and oral shedding.

Keywords: PRRS, vaccination, duration of immunity

Ⅷ-5-058

Protective efficacy of commercial PRRS MLV against dual challenge with type 1 and highly pathogenic PRRS isolates in experimental pigs

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Introduction

This study investigated protective efficacy, reduced viremia and lung lesions, of commercial porcine reproductive and respiratory syndrome virus (PRRSV) modified-live vaccines (MLVs) against challenge with Type 1 and 2 (HP-PRRS) isolates in experimental pigs.

Materials and Methods

Seventy, PRRSV (-), weaned, pigs were allocated to 7 groups of 10 pigs each. Six groups were vaccinated with either: Porcilis® PRRS (Type 1 MLV, MSD AH, The Netherlands), Amervac (Type 1 MLV, Hipra, Spain), Fostera (Type 2 MLV, Zoetis, USA), Ingelvac MLV and ATP (Type 2 MLV, Boehringer Ingelheim, USA) or Prime Pac™ PRRS (Type 2 MLV, MSD AH, The Netherlands), according to manufacturer's direction. A mock vaccinated group served as controls. At 35 days post-vaccination (DPV), all pigs were inoculated intranasally with tissue culture supernatants of Thai field Type 1 PRRSV (SB_EU02, $10^{5.4}$ $TCID_{50}$/mL) and Type 2 PRRSV (ST-US021, HP-PRRSV, $10^{5.2}$ $TCID_{50}$/mL). Serum samples were collected at 3, 5 and 7 days post-challenge (DPC) and quantitatively assayed for Type 1 or 2 PRRSV RNA using RT-qPCR. All pigs were necropsied 7 DPC for macro- and microscopic lung lesions scoring.

Results

PRRSV genomic copies were significantly reduced in all vaccinated groups compared to the mock group from 3 through 7 DPC ($P< 0.05$). Type 1 and 2 genomic copies in serum were lowest in Prime Pac group ($P< 0.05$). Across vaccines, MLV genotype did not influence reduction of viremia. Porcilis pigs had lowest number of PRRSV Type1 genomic copies in serum ($P< 0.05$), while Amervac and Prime Pac pigs had significantly less genomic copies of Type 2HP-PRRSV ($P< 0.05$) compared to other vaccines. Vaccinated pigs had significantly lower lung lesion scores and PRRSV antigens compared to controls ($P< 0.05$). Prime Pac pigs had lowest micro- and macroscopic lung lesion scores ($P< 0.05$) compared to other vaccinated groups.

Conclusion

Modified-live PRRSV vaccines, regardless of vaccine genotype, reduce viremia and lung lesion following dual PRRSV challenge.

Keywords: protective efficacy, porcine reproductive and respiratory syndrome virus, modified-live vaccine, viremia, lung lesion

VIII-5-071

The use of GP5 mosaic T-cell vaccine in a DNA prime vaccinia boost format to cross-protect swine against heterologous PRRSV strains

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Introduction
Design, construct and evaluate T-cell epitope mosaic DNA and vaccinia (VACV) vaccines to improve the breadth and depth of protection towards divergent PRRSV strains.

Materials and Methods
GP5-Mosaic, GP5-WT or vector-control DNA vaccines were administered to 3-4 week-old, PRRSV-free, cross-bred piglets using different delivery systems to ascertain immunogenicity and cross-reactivity (Trials 1 to 3). Briefly, vaccine DNA (500 µg) was administered at day 0 and boosters given once or twice at days 14 or 21. Pigs were challenged at day 35 with VR2332. To test cross-protection (Trial 4), piglets were primed with GP5-Mosaic DNA vaccine and boosted with recombinant GP5-Mosaic VACV (rGP5-MOSAIC-VACV) at day 28. Pigs vaccinated with rGP5-WT DNA and rGP5-WT VACV or empty vector DNA and empty VACV served as controls. Virus challenge was given to separate groups of vaccinated pigs with VR2332 or MN184C. Necropsies were performed at 14 days after challenges.

Results
Antibodies and cellular responses were detected in GP5-Mosaic-vaccinated pigs confirming immunogenicity. Lymphocyte proliferative responses detected in virus-stimulated PBMN cells of GP5-Mosaic-vaccinated pigs were higher than those of controls in both Trials 1&2. In Trial 2, significantly higher levels of IFN-γ mRNA, were detected in GP5-Mosaic-vaccinated pigs as compared to controls. Virus-specific antibodies were higher in GP5-Mosaic-vaccinated animals than in controls in Trials 2&3. The antibodies were shown to neutralize virus. A rapid virus clearance in serum, lower viral loads in tissues, and lower lung lesion scores were recorded in GP5-Mosaic-vaccinated animals compared to controls in Trial 2. In Trial 3, significantly higher IFN-γ mRNA expression was detected in PBMCs from GP5-Mosaic-vaccinated animals upon stimulation with divergent PRRSV strains, while the response was limited to VR2332 in PBMCs from GP5-WT-vaccinated animals. GP5-Mosaic vaccine induced both cellular cross-reactivity and neutralizing antibodies to either VR2332 or MN184C while GP5-WT-vaccinated pigs only responded to VR2332. GP5-Mosaic vaccine reduced viral loads in sera, tissues, BALF, and PAMs in pigs with either virus challenge, and showed lower lung lesion scores than either GP5-WT or vector-control-vaccinated pigs upon challenge with MN184C.

Conclusion
GP5-Mosaic vaccine using DNA-prime/VACV boost regimen conferred cross-protection against heterologous PRRSV strains VR2332 and MN184C in pigs.

Keywords: PRRSV, mosaic T-cell vaccine, VACV, cross-protection

Ⅷ-5-010

Improvement of PRRSV isolation from clinical samples using a ZMAC cell line

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Introduction

Due to high rate of genetic and antigenic diversity of PRRSV, the commercial vaccines are not always effective against field isolates. PRRSV virus isolation (VI) is often conducted to obtain isolates for producing autogenous vaccines. However, PRRSV VI success rate in MARC-145 cells is frustratingly low. Recently, a ZMAC cell line derived from porcine alveolar macrophages becomes available. The objectives of this study were to compare PRRSV VI efficiency in ZMAC and MARC-145 cells and investigate if ZMAC-derived PRRSV isolates grow in MARC-145 cells and vice versa.

Materials and Methods

220 PRRSV-2 and 51 PRRSV-1 PCR-positive clinical samples with various CT values were tested. These included 95 serum, 91 lung, and 34 oral fluid (OF) samples for PRRSV-2, and 21 serum, 8 lung, and 22 OF samples for PRRSV-1. VI was conducted in two cell lines for a head-to-head comparison. Additionally, 84 ZMAC-derived PRRSV-2 isolates were tested in MARC-145 cells and 43 MARC-derived PRRSV-2 isolates were tested in ZMAC cells to evaluate their growth.

Results

PRRSV was not isolated from any OF samples in this study regardless of using ZMAC or MARC-145 cells. Among 186 PRRSV-2 serum and lung samples, 55% and 28% were VI positive in ZMAC and MARC-145 cells, respectively. The success rates of isolating PRRSV-2 from serum and lung samples with different CT values were: 88% *vs* 54% (CT<20), 71% *vs* 36% (20-25), 30% *vs* 8% (25-30), 4% *vs* 0% (30-37) in ZMAC and MARC-145 cells, respectively. Among 29 PRRSV-1 serum and lung samples, 38% and 14% were VI positive in ZMAC and MARC-145 cells, respectively. Only 54% of ZMAC-derived PRRSV-2 isolates grew in MARC-145 cells while 100% of MARC-derived PRRSV-2 isolates grew in ZMAC cells.

Conclusion

Success rate of PRRSV VI from serum and lung samples with CT<30 using ZMAC cells was significantly higher compared to MARC-145 cells. This study clearly demonstrates that ZMAC cells could significantly improve PRRSV VI success rate from clinical samples. It is noteworthy that not all PRRSV isolates obtained in ZMAC cells grew in MARC-145 cells.

Keywords: PRRSV, virus isolation, ZMAC, MARC-145

I -176

Evaluation of a novel PCV + *M. hyopneumoniae* vaccine in swine experimentally challenged with PCV2d and *M. hyopneumoniae* under commercial conditions

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Introduction
Porcine circovirus type 2 (PCV2) is associated with a wide range of clinical presentations known as PCV2 associated disease (PCVAD). Vaccination is essential for herd protection. Most commercial vaccines are based on PCV2a, which is no longer the predominant field strain. This study investigated the ability of a novel, experimental vaccine or current commercial vaccines to protect against dual challenge of *M. hyopneumoniae* and PCV2d under commercial conditions.

Materials and Methods
Pigs (n=880), with no history of vaccination or exposure to *M. hyopneumoniae* or PCV2, were allocated to 1 of 8 vaccine protocols in a generalized randomized block design. Three commercial and one experimental vaccine were given either as one dose at 3 wk of age (Fostera® PCV MH, CircoFlex®+MycoFlex®, Experimental PCV MH), two doses at 3 d/3 wk (Circumvent® PCV-M G2, Experimental PCV MH) or 3 wk/5-6 wk of age (Fostera® PCV MH, Experimental PCV MH). Two weeks after vaccination, pigs were challenged intratracheally with *M. hyopneumoniae* lung homogenate. One week later, pigs were challenged with PCV2d. A subset of pigs (n=24 per treatment) was necropsied 3 wk later and lungs and other tissues were examined for PCVAD lesions. The remaining pigs (n=86 per treatment) were weighed periodically until day 157. Net revenue was determined for culls, lights and full value pigs. Statistical analyses were performed for the primary variables at the 5% level of significance.

Results
Results showed that PCV2d viremia was significantly reduced in vaccine groups compared with saline control ($P \leq 0.05$). CircoFLEX®+MycoFLEX® had significantly higher lung scores than the other vaccine groups ($P \leq 0.05$). Lymphoid depletion and PCV IHC were significantly lower ($P < 0.05$) in vaccine groups compared with saline. Death loss post-challenge in the saline control group was 9.1% (40% had PCVAD), compared with 0.9% to 2.9% in the vaccine groups. The saline group delivered 70.2% full value pigs, while vaccine groups delivered 79.3% to 91.5% full value pigs.

Conclusion
Net revenue modeling showed that vaccines delivered 19.2% to 27.2% greater revenue than saline. Revenue per head placed was $105.09 USD for the saline control. Vaccinates ranged from $125.25 (Circumvent®-M G2) to $133.71 (Fostera® PCV MH).

Keywords: porcine circovirus, vaccine, *Mycoplasma hyopneumoniae,* PCVAD

I -012

No influence of maternal antibodies on piglet serological IgM response to PCV2 vaccination

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Introduction
Several field cases in the Netherlands showed varying proportions of PCV2-IgM positive piglets at 3-5 weeks post vaccination (pv) with Ingelvac CircoFLEX (CF). A possible explanation for the variation in serological IgM results pv is the level of maternal antibodies (MDA), as in some studies apparently MDA interfered with the humoral immune response pv. The aim of the study was to have an indication of the percentage of positive IgM results in the first weeks after CF vaccination, comparing high and low MDA, under field conditions.

Materials and Methods
In a Dutch farm 26 sows parity 1 to 3 were classified according to their serological PCV2 antibody status: IgG positive (high MDA) and IgG negative (low MDA). In every litter 2 piglets of good condition were included and tested serologically at 2, 4 and 9 weeks of age (woa) for PCV2 IgG and IgM (Ingenasa) and by pooled PCR for PCV2. All piglets were vaccinated 1 mL CF at 2.5 woa. After weaning at 4 woa the piglets were placed into one nursery room.

Results
IgG response at 2 days before vaccination was seen in 75% of 'high MDA' piglets and in 4% of 'low MDA' piglets. IgM response at 12 days after vaccination was seen in 50-73% of the piglets and at 50 days after vaccination in 0-4% of the piglets. All samples were tested negative for PCV2 by PCR.

Conclusion
It has been repeatedly demonstrated that CF vaccination is efficacious also at high levels of MDA. The IgG results of the piglets before vaccination reflect the sow's IgG status. After vaccination no difference in IgM response was seen between 'MDA high' and 'MDA low' piglets. In this case we found no influence of level of MDA on the IgM response following Ingelvac CircoFLEX vaccination.

Keywords: maternal antibodies, IgM response, vaccination, PCV2, CircoFLEX

I -194

Genome diversity and multiplex detection assay development for PCV3 and PCV2 viruses

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Introduction
The newly identified porcine circovirus 3 (PCV3) is reported to cause clinical signs similar to that caused by porcine circovirus 2 (PCV2). However, PCV3 genome shares little sequence identity to the PCV2 genome. In this study, we have developed a multiplex real-time PCR assay that can detect and differentiate the majority of the field strains of PCV3 and PCV2. The other objective was to sequence more PCV3 genomes to study the genetic diversity of the PCV3 strains, and to guide the potential modification to existing assays to ensure diagnostic coverage.

Materials and Methods
We have analyzed 1907 available PCV2 full- or near-full genome sequences and designed two assays. Each individual assay can detect 94.8% or 90.5%, and in combination can detect 98.9% of PCV2 strains, including PCV2a, 2b, 2c, 2d and 2e strains. This is a significant improvement to several existing PCV2 assays. Our first PCV3 assay was designed based on the limited 32 genome sequences available at the time of design. With more sequences become available, both from the NCBI GenBank and from our own lab, a second test was designed to overcome the potential problem of primer mismatches to emerging field strains. In combination with our first designed assay it covers all 89 available sequences with 100% coverage. Phylogenetic analysis of the 89 PCV3 full genomes indicated that the largest genetic diversity rate for PCV3 currently is 3.2%. Most of the 37 PCV3 genomes we sequenced were grouped into different clusters with published PCV3 genomes from different geographical locations.

Results
As most of samples we sequenced were collected from the state of Kansas, our data indicated that the mutations in PCV3 strains in the past two years do not show a geographic distribution pattern, and they rather mutated randomly in the genome. The 3.2% mutation rate in the 2,000 bp PCV3 genome represents 64 nucleotide mutations, indicating that the virus is changing.

Conclusion
Continued monitoring the evolution of the virus may be necessary to trace the emerging strains and genotypes of the virus and their distributions. It is also important to modify molecular detection assays accordingly in order to keep assays up to date.

Keywords: porcine circovirus 2, porcine circovirus 3, multiplex PCR, real-time PCR, diagnostics

I -146

Detection of porcine circovirus type 3 (PCV3) and porcine circovirus type 2 (PCV2) in farms vaccinated and non-vaccinated against PCV2

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Introduction
PCV2 is a globally spread pathogen involved in number of diseases (PCVD). Commonly used vaccines against PCV2 are proved to be highly efficacious. PCV3 was discovered in USA in 2016 and subsequently in several other countries, but its role in pig health remains unknown. However, most of the reports described PCV3 in diseased pigs. The aim of the study was to assess the presence of PCV3 and PCV2 in serum samples collected from pig farms of different PCV2 vaccination status.

Materials and Methods
Serum samples (n=798) were obtained from 6 farms vaccinated against PCV2 (VF) and 4 non-vaccinated farms (NVF). Serum samples were collected from 3-21-week-old pigs and sows. Samples were pooled by 4-6 before DNA extraction and tested with real-time PCR for PCV3 and PCV2.

Results
In VF, 19.6% and 3.3% of serum pools were positive for PCV3 and PCV2, respectively. PCV2 was detected only in fatteners (4.2%) and sows (7.1%). PCV3 viremia was found in piglets (12.5%), weaners (9.1%), fatteners (25.0%) and sows (21.4%). PCV3/PCV2 coinfections were detected only in sows and represented 1.1% of all tested pools. In NVF, PCV2 and PCV3 were detected in 56.7% and 20% of pools, respectively. 12.5%, 40.0%, 79.4% and 25.0% of pools collected from piglets, weaners, fatteners and sows were PCV2-positive, respectively. Similarly to VF, PCV3 viremia was the most common in fatteners (29.4%), but it was also detected in weaners (10%) and sows (12.5%). Overall, concurrent PCV3/PCV2 infections were detected in 8.3% of pools.

Conclusion
Vaccination against PCV2 significantly limits PCV2 viremia (3.3% positive pools in VF vs. 56.7% in NVF). The occurrence of PCV3 viremia in VF and NVF was similar (19.6% vs. 20%, respectively). It can be assumed that PCV2 vaccination has no impact on PCV3 infection. Also, it does not seem that the intensive PCV2 circulation in NVF facilitates PCV3 infection. Further studies are needed to establish correlation between the presence of PCV3 and other common pathogens in pig farms.
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Keywords: PCV3, PCV2, real-time PCR

I -135

Development of antibody titers after porcine circovirus vaccination in piglets with different levels of maternally-derived antibody

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Introduction
Porcine circovirus type 2 (PCV2) is a causative agent for porcine circovirus-associated disease (PCVAD). It has been reported that maternally-derived antibody (MDA) against PCV2 can interfere with the generation of a humoral immune response in vaccinated piglets, although such piglets may still develop immunological memory and be protected from challenge. The objectives of this study were to investigate the antibody responses of piglets after PCV2 vaccination under various MDA conditions, and the correlation of two measurement methods of antibody titer.

Materials and Methods
Seven pregnant sows were administered PCV2 vaccine (Fostera® PCV, Zoetis) 3weeks before farrowing to stimulate high maternal antibody concentrations. Thirty-six piglets from these sows were divided into 5 groups (T1 to T5). T1 to T4 had 8 piglets per group and were administered Fostera® PCV at different weeks of age, 3 (T1), 5 (T2), 7 (T3) and 9 (T4). T5 comprised the remaining 4 piglets and acted as negative control. Blood samples were collected at 1, 2 and 3 weeks of age and every 2weeks thereafter until 15 weeks. Antibody titers were measured by indirect immunofluorescence assay (IFA) and commercial ELISA kit (SERELISA®, Zoetis). PCV2 DNA was assessed by real-time PCR.

Results
In the T1 group, there was no clear rise of antibody titer. In the T2 to T4 groups, there were the clear increases in antibody titers after vaccination. In the T5 group, antibody titer decreased gradually. PCV2 DNA was negative in all samples. The correlation coefficient between IFA antibody titer and ELISA values was 0.7. The piglets with MDA titers below 400 (IFA) or 600 (ELISA) at the time of vaccination showed a clear increase in antibody titer following vaccination in this study.

Conclusion
Antibody titers against PCV2 measured by IFA and the SERELISA® ELISA kit can be correlated with each other. In the case of dams with high levels of MDA a clear post-vaccination increase in antibody titers against PCV2 can be expected in piglets with titers below 400 (IFA) or 600 (ELISA) at the time of vaccination.

Keywords: porcine circovirus, maternally-derived antibody, vaccine, immunofluorescence assay, enzyme-linked immunosorbent assay

I -141

Comparison of serum PCR assay for porcine circovirus 2 with histopathology in slaughter pigs from the Philippines

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Introduction

Porcine circovirus type 2 (PCV2) is widespread in pigs. The conventional diagnostic approach requires assessment of three aspects - clinical signs, characteristic histopathological lesions in relevant tissues, and confirmed presence of the virus in the lesions. We have recently established an assay (developed by others) that can quantify PCV2 levels in serum. The potential of a serum test to replace the conventional diagnostic tools is yet to be established. The objectives of this work were to determine the presence of typical PCV2-associated lesions in the spleen and mediastinal lymph nodes of pigs examined for the presence of bacterial respiratory pathogens in the Philippines and to compare the results of the histopathological examination with the levels of PCV2 in the serum of the animals.

Materials and Methods

The tissues subjected to histopathological examination were from 156 pigs that came from eight farms (4 farms in each of Bulacan and Pampanga provinces) collected as part of a previous study. The histopathological examination was performed using standard protocols in place at the Animal Disease Diagnosis and Reference Laboratory. The qPCR results were available for the same pigs (see related poster).

Results

Of the 156 spleens examined, only four animals showed severe lymphocyte depletion. Of the 156 mediastinal lymph nodes (MLN) examined, only seven pigs had severe lymphocyte depletion. No pig showed severe histiocytic inflammatory infiltration in either spleen or MLN. One pig had severe lymphocyte depletion in both spleen and MLN and this pig had more than 10^6 DNA copies of PCV2 per milliliter of serum. The only other animal with more than 10^6 DNA copies of PCV2 per milliliter of serum had moderate lymphocyte deletion in both spleen and MLN. A total of 44 other animals had moderate lymphocyte depletion in spleen and MLN but all had less than 10^6 DNA copies per milliliter of serum. Further work is required to determine if PCV2 levels in serum can be used to diagnose PCV2 disease in the Philippines setting.

This work is supported by ACIAR and PCAARRD.

Keywords: PCV2, histopathology, Philippines

II-213

Modified method for the minimal inhibitory concentration (MIC) determination of *Lawsonia intracellularis* without cell culture system using propidium monoazide-qPCR

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Introduction
Lawsonia intracellularis (LI) is an intracellular entero-pathogen causing two main clinical manifestations in pigs: an acute hemorrhagic form often called proliferative hemorrhagic enteropathy (PHE), and a more chronic proliferative form often referred as proliferative intestinal adenomatosis (PIA). Up to now, antimicrobial therapy remains to be the only available option for treatment. Tiamulin, tylosin, lincomycin, and chlortetracycline have been commonly recommended in the field. Oxytetracycline, valnemulin, doxycycline, josamycine, and leucomycin were also known as effective according to field experiences, not from the exact in vitro antimicrobial susceptibility testing (AST). However, AST could not be easily conducted for LI because it requires complicated cell culture system and particular atmosphere for its growth. Propidium monoazide (PMA) is a photoreactive DNA-binding dye that inhibits PCR amplification by modifying chromosomal DNA. PMA is intercalated into the dead bacteria and covalently cross-linked to bacterial DNA, which strongly inhibits amplification. Therefore, the aim of this study was to modify how to determine the minimal inhibitory concentration (MIC) of LI without cell culture system using PMA-qPCR.

Materials and Methods
The four novel LI field isolates were obtained from hemorrhagic intestines from a finisher pigs (CBNU001, CBNU002, and CBNU006) and lactating piglets (CBNU004) with PHE in 2013 to 2017. The isolates were prepared in IEC-18 cells and harvested and then, the AST was determined by conventional exteracellular minimum inhibitory concentrations (exMIC)s and PMA-qPCR against isolates.

Results
The results showed that CT value of PMA-qPCR assay was correlated with patterns of conventional exMIC in LI antimicrobial activity test.

Conclusion
Therefore, this modified method using PMA-qPCR for determining the exMIC of LI without cell culture system was useful to evaluate antimicrobial susceptibility and to save time to detect.

Keywords: *Lawsonia intracellularis*, proliferative enteropathy, minimal inhibitory concentration, propidium monoazide

II-221

In vitro comparison of antimicrobial activities against three isolates of *Lawsonia intracellularis* generated from pigs in Thailand

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Introduction

Lawsonia intracellularis (LI) a Gram-negative obligate intracellular organism, is the causative agent of porcine intestinal adenomatosis (PIA) as well as proliferative haemorrhagic enteropathy (PHE) in nursery and adult pigs, respectively. This organism can cause major economic loss in swine production worldwide. Only few pure cultures of LI have been successfully isolated worldwide. The aims of this study were to isolate *L. intracellularis* from field samples in Thailand and to determine the *in vitro* antimicrobial activities against these isolates.

Materials and methods

Two cases of PIA and one case of PHE lesions were collected from pigs from different farms in the western region of Thailand. All samples were strongly PCR positive for LI. Subsequently, the attempted isolations of the bacteria were performed using McCoy cells. The growth of bacteria was monitored by immunoperoxidase assays with specific polyclonal antibody. After the isolates were purified and reached 100 % growth of heavily infected cells (HIC), the minimum inhibitory concentration (MIC) test was performed for both intracellular and extracellular activities of antimicrobials at concentration ranging from 0.125 to 128 µg/mL. The antimicrobials included amoxicillin, bacitracin, carbadox, chlortetracycline, enrofloxacin, gentamicin, lincomycin, colistin, spectinomycin, sulfamethazine, trimethoprim, tylosin, tiamulin, and valnemulin.

Results

All three strains of LI were successfully isolated with 100% HIC at passage 4 to 5 and all were continuously grown up through passage 10. For both intracellular and extracellular MIC testing, carbadox, tiamulin and valnemulin were the most active antimicrobials against all three Thai LI isolates with MICs ranging from 0.125 to 8 µg/mL. Tylosin displayed intermediate activity with MICs ranging from 2 to 64 µg/mL. Finally, bacitracin, chlortetracycline, colistin, gentamicin, lincomycin, spectinomycin, sulfamethazine and trimethoprim showed the lowest activities with the various MICs ranging from 64 to >128 µg/mL.

Conclusion

This is the first report on successful cultivation and maintenance of LI *in vitro* in Thailand and corresponding MIC testing. Our data extend the antimicrobial MIC information for LI. We confirm that each LI isolate has a unique antimicrobial sensitivity pattern. The results indicate that carbadox, tiamulin and valnemulin are the most active antimicrobials against Thai LI isolates.

Keywords: *Lawsonia intracellularis*, proliferative enteropathy, antimicrobial sensitivity, MIC, Thailand

II-194

Improvement of pork odor using vaccination against *Lawsonia intracellularis*: a case report

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Introduction
Skatole and indole accumulated in fat tissue are known to be the causative substance of unpleasant pork odor. Pork produced from pigs of a Japanese farm experiencing diarrhoeic symptoms due to *Lawsonia intracellularis* (*Li*) infection in late fattening had odor issues. We report here the results of implementing vaccination, including the effect of skatole and indole concentrations in backfat.

Materials and Methods
Black tarry diarrhoea and sudden death were observed in late fatteners in a 500-sows farrow-to-finish farm indicated proliferative hemorrhagic enteropathy (PHE). A hose-like thickening of ileum mucosa covered with pseudomembrane was found at necropsy confirming the clinical diagnosis. Serum samples were tested for *Li* antibodies (bioScreen Ileitis antibody-ELISA) confirmed seroconversion at 120 days old. Therefore, vaccination with Enterisol® Ileitis was implemented at 40 days old. Around the same time, the pork sales destination reported that their customers brought complaints of pork odor. This pork was from the batch having the PHE problems. Seven carcass samples of the batch were submitted to NH Foods Ltd. R&D Center for determination of skatole and indole concentrations in backfat. Skatole concentration was 0.04-0.53 µg/g (average 0.22 µg/g), three samples (42%) exceeded the sensory threshold of 0.2 µg/g. Indole concentration was less than sensory threshold of 0.3 µg/g, ranging 0.03-0.15 µg/g (average 0.09 µg/g).

Results
Five months after implementing vaccination when the vaccinated pigs were marketed, skatole and indole concentrations of backfat were measured using six samples, and *Li* antibody test was performed again. As expected, serology confirmed that *Lawsonia* was still present on the farm. Average concentrations of skatole and indole were 0.07 µg/g and 0.02 µg/g, respectively, a decrease of 68% and 78% and compared to before vaccination. No samples exceeded sensory threshold.

Conclusion
Skatole, by-product of microbial breakdown of tryptophan in the intestine, has strong odor. We hypothesized that intestinal lesions of PHE led to increased abnormal fermentation, which caused elevetad skatole production in the intestine. Consequently, nondegraded skatole deposited in fat was elevated. In this case, vaccination against *Li* infection improved clinical symptoms as well as pork odor.

Keywords: *Lawsonia intracellularis*, pork odor, skatole, vaccination

II-184

A technique to monitor plant-based feed additive's efficacy in controlling *Lawsonia intracellularis* infections in pigs

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Introduction

This experiment was aimed at the evaluation of the efficacy of a plant-based feed additive (Patente Herba® Plus) on *Lawsonia intracellularis*.

Materials and Methods

In fecal samples of naturally (subclinically) infected weaned pigs *L. intracellular* is was quantified using real-time quantitative Polymerase Chain Reaction (qPCR). A total of 12 (6 pigs in treated and 6 in control group) weaners, aged seven weeks, were randomly separated into 12 individual boxes where physical contact with other pigs was prevented. The only difference was that the treated group received the plant-based additive in at the concentration of 2 kg/t of feed. Feces was sampled individually on days 0, 14 and 28 and tested in qPCR assay. Due to the lack of clinical symptoms the presence of *Escherichia coli*, *Salmonella* spp., *Brachyspira hyodysenteriae* and *B. pilosicoli* was excluded.

Results

Results of qPCR, expressed as cycle-threshold (Ct) values, identified and revealed the presence of *L. intracellularis* subclinical infection in all pigs on day 0. However, on day 14 Ct values were statistically higher in the treatment group than in the control ($P < 0.05$). A similar trend was observed on day 28. Data obtained in the current research have proved that the quantification of *L. intracellularis* in fecal samples with the real-time qPCR test may be useful for assessing the efficacy of products having the potential to control *L. intracellularis* as well as for the diagnosis of sub-clinical proliferative enteropathy (PE). PE results in ineffective digestion and reduced nutrient absorption in pigs, which lead to poor production and financial losses.

Conclusion

Therefore, reliable techniques such as qPCR for monitoring *L. intracellularis* are becoming imperative in increasing awareness about PE at farms.

Keywords: *Lawsonia intracellularis*, proliferative enteropathy, plant-based feed additive, pig

II -095

Automated genome sequence interrogation to support surveillance, tracking and control of *Brachyspira hyodysenteriae* infections

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Introduction
*Brachyspira hyodysenteriae*is the commonest etiological agent of swine dysentery. This study aimed to develop an on-line search tool to interrogate whole genomic sequence data from these bacteria to establish the Multilocus Sequence Type (MLST), presence or absence of 332 putative virulence and lifestyle genes (La et al. 2016), and single nucleotide polymorphisms (SNPs) that may be related to resistance to antimicrobials. The genome sequences of 42 strains of *B. hyodysenteriae* isolated from pigs in Germany (n=23) or Australia (n=19) were accessed from the Brachyspira genome sequence database at Murdoch University. The sequences were obtained through next generation sequencing using the Illumina platform. The sequences previously had been manually interrogated to establish the MLST sequence type (ST) and presence of virulence genes.

Materials and Methods
The program was developed using C++ and R, and was applied to the genomes. MLST data were obtained from the PubMLST database (**https://pubmlst.org/**). Putative virulence genes were reported by Black et al. (2015) and SNPs associated with resistance to doxycycline, tylosin, lincomycin and tiamulin were identified (Karlsson et al. 1999; Mahu et al. 2017).

Results
The program correctly identified the 42 strains as belonging to 18 different ST, with between 206 and 302 putative virulence genes. Thirteen strains (1 from Australia and 12 from Germany) had a G1058C mutation on the 16S rRNA gene associated with resistance to doxycycline. Twenty-one strains (11 from Australia and 10 from Germany) had an A2058T mutation on the 23S rRNA gene associated with tylosin and lincomycin resistance, and 8 strains (6 from Australia and 2 from German) had an A2058G mutation associated with tiamulin resistance.

Conclusion
As whole genomic sequencing becomes routine, this automated approach to extracting relevant data represents a rapid and simple method to support local and national control of *B. hyodysenteriae* infections. The program is being fine-tuned to improve identification of relevant genes and SNPs, and will be made available for research and diagnostic purposes.

Keywords: *Brachyspira hyodysenteriae*, genome, MLST, virulence, antimicrobial resist

II-073

Identification of a new pleuromutilin resistance gene in *Brachyspira hyodysenteriae*, aetiological agent of swine dysentery

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Introduction
The aim of this work was to investigate antimicrobial resistance in *Brachyspira hyodysenteriae* the aetiological agent of swine dysentery, a globally distributed disease that causes significant economic loss and has a profound impact on pig health.

Materials and Methods
We undertook whole genome sequencing and antibiotic susceptibility testing of 34 UK field isolates and 3 ATCC control strains to investigate tiamulin resistance, a widely used antibiotic for treatment of swine dysentery.

Results
Genome-wide association studies identified a new pleuromutilin resistance gene, termed *tva*(A) (*t*iamulin *v*alnemulin *a*ntibiotic resistance), encoding a predicted ATP-binding cassette sub-family F protein. *In vitro* culture of isolates in the presence of inhibitory or sub-inhibitory concentrations of tiamulin showed that *tva*(A) confers reduced pleuromutilin susceptibility that does not lead to clinical resistance but facilitates the subsequent development of higher-level clinical resistance via mutations in chromosomal genes encoding ribosome-associated functions. Molecular epidemiological investigation of isolates obtained over time during two episodes of swine dysentery on the same farm indicated that *tva*(A) contributes to development of tiamulin resistance in the same manner *in vivo* as seen experimentally *in vitro*. Furthermore *tva*(A) raised the tiamulin mutant prevention concentration above reported *in vivo* tiamulin concentrations obtained when administered at certain doses.

Conclusion
This work has identified a new marker for pleuromutilin resistance and provides evidence to inform treatment regimes and reduce the development of resistance to this class of highly important antimicrobial agents.

Keywords: *B. hyodysenteriae*, swine dysentery, tiamulin, pleuromutilin, resistance

II -096

Mobile colistin resistance gene *mcr*-5 in porcine *Aeromonas hydrophila*

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Introduction

The mobile colistin resistance genes *mcr* has heralds the breach of polymyxins, one of the 'last-resort' drugs against for multidrug-resistant gram-negative bacteria. To date, five plasmid-mediated colsitin resistance genes, *mcr-1*, *mcr-2*, *mcr-3*, *mcr-4*, and *mcr-5*, were identified, with *Enterobacteriaceae* being the predominant hosts. Among them the *mcr-5* gene has only been found in *Salmonella spp.* from poultry and animal-derived food products in Germany. So here we characterize the mobile colistin resistance gene *mcr-5* in *Aeromonashydrophila* from backyard pigs in rural areas of China.

Materials and Methods

A total of 336 faecal samples were collected from backyard pigs from 194 households across 12 villages in rural areas of Shandong Province, China, in August 2017. All the samples were directly tested for the presence of *mcr-5* by PCR assay. The phenotypic antimicrobial susceptibility profiles of the *mcr-5*-positive isolates were determined using the broth dilution method. The genomic location and transferability of *mcr-5* were analysed by S1-PFGE with Southern blotting and natural transformation, respectively. One strain isolated from a *mcr-5*-positive sample was subjected to WGS, and the stability of the *mcr-5*-harboring plasmid over successive generations was examined by sub-culturing.

Results

One *mcr-5*-positive *A. hydrophila* isolate (I064-2) showing resistance with colistin MIC 4 mg/L was isolated from a backyard pig faecal sample. *Mcr-5* was located on a 7915-bp plasmid designated pI064-2, which could naturally transform into a colistin-sensitive *A. hydrophila* strain of porcine origin, and mediated colistin resistance in both original isolate and its transformants. PI064-2 is stable in the wild-type parent, while it was only maintained in the *mcr-5*-positive transformant for 20 generations without the pressure of colistin. The plasmid backbone (3790 bp) of pI064-2 showed 81% nucleotide sequence identity to the corresponding region of ColE2-type plasmid pAsa1 from *Aeromonas salmonicida*, while its similar replication primases are widely distributed among aeromonads, *Enterobacteriaceae*, and *Pseudomonas* species.

Conclusion

This is the first identification of colistin resistance gene *mcr-5* in an *A. hydrophila* isolate from the faeces of a backyard pig. *Mcr-5* is expected to be able to disseminate among different bacterial species and genera.

Keywords: *mcr-5*, *Aeromonas hydrophila*, backyard pigs

II-219

The occurrence and characteristics of *Clostridium difficile* isolates from pig breeds in the Czech Republic

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Introduction
The anaerobic bacteria *Clostridium difficile* is one of the most common causes of nosocomial diarrhoeas (CDI) in humans after antibiotic treatment. The CDI is associated with high mortality and economic burden. The occurrence and clinical importance was also recognized in several animal species and well-studied has been among pigs. Because the lack of information on the presence of *C. difficile* in animals and in the breeding environment in the Czech Republic, we aimed to determine the occurrence of *C. difficile* in pigs in the Czech Republic with detail characterisation of isolates by capillary electrophoresis-ribotyping, the presence of toxin genes (*tcdA*, *tcdB*, *cdtA*, *cdtB*).

Materials and Methods
For this purpose, 178 faeces from sucking piglets with diarrhoea and their mothers were cultured anaerobically on selective medium for *C. difficile* (Oxoid). A total of 58 (56 piglets, 2 sows) samples from 10 farms were *C. difficile* positive. All 58 isolates were further characterized.

Results
PCR ribotyping revealed 10 different profiles (011 n=1, 014 n=1, 033 n=10, 049 n=4, 078 n=24, 078-like n=5, 126 n=1, 150 n=7, 413 n=4, and new n=1). RTs 078, 126 and 413 carried genes for production of all three toxins (A, B, binary toxin), RT 033 isolates were only binary toxin genes positive. The remaining profiles (011, 014, 049, 150 and new) were toxigenic (A, B). In the Czech breeds, RT 078 was the most common ribotype, followed by 033 and 049.

Conclusion
According to recently published studies, RT 078 currently dominates in other European. In almost every farm (with one exception), *C. perfringens* was also cultured along with *C. difficile*. These two species on the contrary didn't compete. Impact of with *C. difficile* and *C. perfringens* co-infection may emphasize and can explain mutual *C. perfringens* therapy and vaccination strategy failure.

Keywords: *Clostridium*, *Clostridium difficile*, diarrhea, ribotype

II-129

Analysis of microbial community structure in the digestive tract of Rongchang pigs of different age

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Introduction
In this study, 5 healthy Rongchang pigs with each stage of lactation (12 d), weaning (60 d), fattening (90 d) and adult (24 months) were selected as experimental animal. The contents of stomach, duodenum, jejunum, ileum, cecum, colon and rectum of each pig were collected as test samples. High-throughput sequencing of V3-V4 variable regions of 16S rDNA of the bacterial flora in 140 collected samples were performed on a Miseq 2×300 sequencing platform, and only quality-controlled sequencing sequences can be used for subsequent bioinformatics analysis.

Results
As a result, a total of 2755101 effective DNA sequences with an average length of 40835bp were obtained successfully. The data showed that the rich rarefraction curves of each sequencing sample tend to plateau, however, the Shannon rarefraction curve of corresponding sample reached a flat. These results suggested that the amount of sequencing data represent the information of most microbial species in the habitats of porcine digestive tract. The analysis of alpha diversity showed that there were significant difference ($P< 0.05$) in ACE indexes and Chao1 indexes in similar habitat of pigs at different ages, while no significant difference were observed on Shannon indexes and Simpson indexes.

Conclusion
The findings in the study might suggest that the increase of age, accordingly, the microbial community abundance in similar habitats of porcine digestive tract increased. However, the flora diversity did not increase significantly. The results indicated that the colonization of the gut microbiota has been completed during the pig feeding and exposure to the environment. Finally, based on the analysis of distribution barplot in the phylum level, it can be found the microbial communities in the samples consisted mainly of *Firmicutes*, *Proteobacteria*, *Bacteroides*, *Chlorobi*, *Verruciformes* and others. And among them the abundance of *Proteobacteria, Bacteroides, Chlorobi* and V*erruciformes* do not change significantly in different habitats of different age pigs. Besides, clustering heatmap showed that the flora structure change gradually with the different sampling habitats and the age of pig.

Keywords: pigs of different ages, habitats, microbial community, heatmap

II-075

MEFA (multiepitope fusion antigen), a structural vaccinology approach for vaccines against porcine post-weaning diarrhea (PWD)

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Introduction

Porcine post-weaning diarrhea (PWD) is a major disease that causes significant economic losses to swine producers worldwide. Currently there are no effective vaccines against PWD. Enterotoxigenic *E. coli* (ETEC) strains producing K88 or F18 fimbriae and enterotoxins including heat-labile toxin (LT), heat-stable toxins (STb, STa) and occasionally shiga toxin Stx2e are the primary cause of PWD. An effective PWD vaccine needs to induce antibodies to block adherence of K88 and F18 fimbriae but also to neutralize enterotoxicity of LT, STs and perhaps Stx2e toxins. Clearly, virulence heterogeneity becomes a major challenge in PWD vaccine development.

Materials and Methods

Here we report a novel structural vaccinology strategy, MEFA – multiepitope fusion antigen, to develop a broadly protective PWD vaccine. This structure-defined MEFA technology, that combines principals of computational biology, structural biology, epitope vaccinology and structural vaccinology, identifies a backbone antigen and presents neutralizing epitopes from all virulence determinants to induce broadly protective antibodies. By selecting a non-toxic LT mutant as the backbone and presenting neutralizing epitopes of K88 and F18 fimbrial adhesins and also STa, STb and Stx2e toxins to mimic their native antigenic propensity, we constructed a single adhesin-toxin MEFA molecule to induce antibodies protecting against K88 and F18 fimbrial adherence and enterotoxicity of all four ETEC toxins.

Conclusion

A vaccine derived from this adhesin-toxin MEFA can induce broad antibody responses and effectively protects against PWD. Additionally, this MEFA structural vaccinology strategy can be applied universally for developing multivalent vaccines broadly against other heterogeneous pathogens and different diseases.

Keywords: PWD, vaccine, structural vaccinology, MEFA

II-060

Innovative and simple method to detect Verotoxigenic *Escherichia coli* (VTEC) by combining the pig oral fluid and the FTA® technology in a qPCR assay

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Introduction
The verotoxin encoded by the *vtx2e* gene in VTEC (*vtx2e*$^{+}$ *Escherichia. coli*) is responsible for the clinical signs and lesions of edema disease (ED) in pigs. The present work aimed to elucidate firstly, if oral fluid (OF) can be used to detect VTEC by qPCR, and secondly if *vtx2e*$^{+}$ OF could be fixed in FTA cards and then be analyzed without losing the assay performance characteristics.

Materials and Methods
The qPCR was optimized to specifically detect VTEC within a diverse panel of bacteria and viruses of porcine origin. Then, the assay was used in a first comparative study in which 217 growing pigs housed in a fattening unit (16 pens) with VTEC circulation, were analyzed collectively by OF (1 per pen: 14 pigs each), and individually by rectal swab (RS) qPCR. A second study compared the performance of the qPCR assay using 28 VTEC-positiveliquid OF, and the same samples fixed in FTA Elute cards. Agreement between sampling methods (OF *vs* RS) and sample nature (liquid *vs* FTA-fixed) was assessed by the *t-test*, on the basis of the semi-quantitative (CT value) and qualitative (+/-) qPCR outputs.

Results
Results from the collective (OF) *vs* individual (RS) sampling methods, showed no significant differences (P<0.001) in average CT values. However, OF was more sensitive (100% positive pens) than RS (75% positive pens) classifying pens as positive or negative. Finally, the qPCR detected the VTEC-positive OF, regardless of whether they were liquid or fixed in FTA.

Conclusion
To the author's knowledge, this is the first report on the use of OF to confirm VTEC infection in pigs. Even more, it is demonstrated that OF samples can be tested for the presence of the *vtx2e* gene in the liquid form upon arrival in the laboratory, or fixed on site on FTA cards and stored for latter submission and analysis, without undergoing denaturation. This method facilitates the handling of samples and reduces costs.

Keywords: VTEC, qPCR, edema disease, pig

II-169

The effects of probiotics in pigs on host responses and microbiota composition and their putative role to prevent *Escherichia coli* infections

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Introduction

The objective of this study is to evaluate the potential of probiotics in pigs to improve immune competence in order to make piglets more resistant to disease and prevent use of antibiotics.

Materials and Methods

To study this, three groups of piglets were treated daily with probiotic strains for a period of 8 days. Subsequently, the intestinal tract was sampled for evaluation of epithelial integrity, microbiota composition, and transcriptional host responses. All piglets were clinically healthy during the experiment, and no morphological changes in epithelial integrity of the intestine were observed. Microbiota composition of groups treated with probiotic lactobacilli changed significantly, revealing that administered lactobacilli were among the most predominant species residing in the jejunum. Gene expression of innate immune genes was determined in intestinal scrapings of lactobacilli treated animals as well as control animals. Expression of IL-6, TNF-alfa, IL-10 and NF-kappa-B was significantly decreased in *Lactobacillus plantarum* treated piglets, demonstrating *L. plantarum* administration elicited transcriptional modulation of innate immune genes. To determine whether this change could potentially contribute to prevention of *E. coli* infections, *in vitro* cell models were used. Intestinal porcine epithelial cells were used as model system. Cells were treated with lactobacilli and subsequently infected with *E. coli*.

Results

Gene expression analysis showed that *E. coli* infection of IPEC cells induced strong pro-inflammatory responses similar to the one observed in vivo, whereas lactobacilli did not induce strong responses. It was shown, that depending on the MOI of lactobacilli, these proinflammatory immune responses to *E. coli* could be dampened by pre-treatment with lactobacilli. This suggests that lactobacilli might be capable of dampening the host responses to *E. coli*, thus causing less disease. However, this has to be tested in vivo to confirm that probiotics can reduce *E. coli* disease.

Conclusion

Taken together, these studies demonstrate that treatment of pigs with probiotics can induce immunomodulatory responses in the intestine *in vivo*. *In vitro* it is shown that probiotics can ameliorate the proinflammatory responses to *E. coli*. Further research is required to demonstrate effectivity against *E. coli in vivo*.

Keywords: probiotics, *Escherichia coli*, microbiota

II-107

Compounds of organic acids, cinnamaldehyde and Permeabilizing Complex™ (PC) impact on growth performance and modulation of gut microbiome in weaning piglets

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Introduction
The aim of the project was to examine the ability of a mixture (ACPC) of organic acids (formic, acetic and propionic), cinnamaldehyde and Permeabilizing Complex™ (PC) to improve growth performance and also impact on intestinal microbiome in weaning piglets. Growth performance as well as the pH in the gastro-intestinal tract (GIT), microbial population in the ileum and villus height of jejunum were determined.

Materials and Methods
Ninety-six weaning pigs were assigned to three treatments and fed commercial diets. The negative control group diet contained no feed additives, whereas the positive control group was supplemented with antibiotics of colistin (100 g/t) and chlortetracycline (100 g/t). The trial group was fed ACPC (1 kg/t).

Results
Results showed that at the 56th day of trial, body weight, average daily gain and feed intake were higher in the trial group compared with the two control groups. The pH in the stomach of pigs fed the negative and positive control diet was similar (4.28 vs. 4.30), while pH in stomach of pigs fed the diet supplemented with ACPS was significantly (P<0.05) lower compared to the stomach pH of pigs in the negative and positive control groups. Microbial analysis showed that the number of *E. coli* and *Salmonella typhimurium* in the ileum of pigs were reduced (P<0.05) in the groups fed ACPC and antibiotics in comparison with the negative control. Counts of *Lactobacilli* and *Bifidobacteria* in the ileum were higher (P<0.05) in the trial group than in the other two groups. The villus height in the jejunum was greater in the positive control group and in the trial group in comparison with the negative control (P<0.05).

Conclusion
These results indicated that the use of organic acids based product improved growth performance in piglets by changing the intestinal micro-ecological environment.

Keywords: weaning pigs, organic acids, growth performance, gut microbiome

Ⅱ-188

The diagnosis and control of one acute case of suckling piglets related to *E. coli* infection

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Introduction
E. coli is one of the important pathogen for suckling piglets with the typical clinical signs of diarrhea and dehydration, which is sometimes responsible for high morbidity and mortality. The treatment efficiency of one acute case of suckling piglets related to *E. coli* infection was highly correlated to medication routine.

Materials and Methods
The 4-7 day-old suckling piglets in one GP farm with 1300 sows inventory showed high percentage of acute death from Sep 17th, 2017. The body condition of the most acute death piglets was very good but the area around dead piglets' neck were significantly swollen. Sick piglets that were unwilling to stand up or move showed high possibility of mortality(>60%). Acute death could happen at the beginning of clinical signs. 40% of litters had yellowish and watery diarrhea. The sows of sick litters did not show significant clinical signs with normal body temperature. But the body temperature of more than 90% sick piglets was lower (36-37℃) than normal. Until Sep 21th, 191 suckling piglets were dead. Mortality was 5.13%. The most significant lesions were edema in the subcutaneous tissue of neck and inguinal, kidney and mesentery. Spleen was a little bit of swollen. Lungs, livers and tonsils were normal. The following injection was used including amoxicillin, ceftiofur, enrofloxacin, gentamycin and draxxinas treatment of the case. But the results of these injection treatment were poor.

Results
The results of bacteria isolation confirmed the *E. coli* infection. We changed the medication routine from injection to oral medication. The results showed that oral medication of amoxicillin and penicillin were very efficacious. The case stopped in 7 days.

Conclusion
E. coli infection of piglets could lead to high acute mortality. The case meant that the medication routine made the big difference in some case, especially with the bacterial infection of intestine.

Keywords: suckling piglets, acute death, edema, medication routines

II -058

Ssment of antibody response and efficacy of vaccines against neonatal diarrhoea by *E. coli*

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Introduction
Neonatal *E. coli* enteritis results in mortality and curative treatments. Sow vaccination is an effective prevention through transfer of antibodies via colostrum. This study compares F4ab, F4ac, F5, F6, LT antigen seroconversion in gilts vaccinated with either Porcilis® ColiClos (PC), vaccine N (+F41 ag) vaccine E (+F18 ag). Protection was evaluated in piglets by clinical and faecal scoring, and antibiotic treatment rates.

Materials and Methods
In a 4,000 sow farm with F4+ *E. coli* neonatal diarrhoea, 92 gilts were vaccinated with one of the 3 vaccines according to the leaflet. Blood sampling was done at 1st, 2nd vaccination and farrowing, and antibody response against F4, F5, F6 and LT was measured. The litter general health, appetite, faecal consistency and composition were scored from 0 (normal) to 3 (ill, not suckling, watery, blood/casts), so a low score means a better result. Litter diarrhoea treatments were scored.

Results
Serology: All vaccines induced antibodies against the measured antigens. PC induced significantly higher F6 and F5 titers than vaccines N and E, while titers were numerically higher for the other antigens with the exception of F4ac (for vaccine E). Protection: Litter scores for general health and faecal consistency of the PC and vaccine N in gilts were significantly lower (=better) than in the litters when compared to vaccine E. In PC vaccinated litters, scores for appetite were significantly lower (=better) than in vaccine E litters. Faecal composition was considered fairly normal as mucus or blood was barely observed. Diarrhea treated litters: PC: 38%. Vaccine N: 52%. Vaccine E: 68%

Conclusion
All vaccines induced an increase in antibody titers against the vaccine antigens. Porcilis® ColiClos induced the highest titer against several of the antigens, which may explain the better general health, appetite, and faecal consistency scores and the numerically lower litter treatment percentage.

Keywords: *Escherichia coli*, colostral immunity, Porcilis® ColiClos

II-028

Vaccination with a live bivalent *E. coli* F4/F18 vaccine for the prevention of F18-ETEC post-weaning diarrhea

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Introduction
Post-weaning *Escherichia coli* diarrhea (PWD) remains a major cause of economic losses for the pig industry. PWD, caused by enterotoxigenic *E. coli* (ETEC), provokes mild to severe watery diarrhea (5-10 days post-weaning). Most common adhesins on ETEC from PWD are the fimbriae F4 (previously called K88) and F18. Recently, an oral live bivalent *E. coli* F4/F18 vaccine (Coliprotec® F4/F18; Prevtec Microbia) is available on the European market, which reduces the impact of PWD provoked by F4-ETEC and F18-ETEC. The objective was to compare technical results of *E. coli* F4/F18 vaccination with previous standard therapeutic approach under field conditions.

Materials and Methods
An 800-sow farm (weaning at 21 days) with diagnosed problems of PWD due to F18-ETEC was selected. Piglets were vaccinated at 18 days with the oral live bivalent E. coli F4/ F18 vaccine. At weaning, no standard group medication (antibiotics) was applied for prevention of PWD. Several performance parameters were collected before (n = 3 groups) and after implementation of the vaccination (n = 5 groups): time in nursery, mortality and medication use (TI_{100}) in the nursey phase and days to slaughter in the fattening phase.

Results
Oral *E. coli* F4/F18 vaccination significantly reduced the mortality (4.3% to 1.9%; $P<0.05$) and the TI_{100} by 80% in the nursery. Finisher vaccinated pigs were slaughtered 7 days earlier at the same end body weight. Production parameters were identical before and after the vaccination.

Conclusion
The live *E. coli* F4/F18 vaccination against PWD has led to similar technical performance parameters, in combination with a significant reduction in mortality and medication use in the nursery phase and reduction of number of days in the fattening. In conclusion, control of PWD through vaccination is a good option in order to prevent piglets from the negative clinical outcomes of F18-ETEC infection during the post-weaning period with additional effect on finisher pig performances.

Keywords: *Escherichia coli* F18, Coliprotec F4/F18

II -064

Comparing post-weaning mortality in pigs born from sows vaccinated with two different *coli-clostridia* combination vaccines

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Introduction
Among the most common reasons of mortality and antibiotics used post-weaning are *E. coli*-associated diseases like post-weaning diarrhea (PWD) and edema disease (ED). Recently a novel *Coli-Clostridia* combination vaccine, Entericolix®, containing F4 and F18 *E. coli* fimbrial antigen, was registered in the EU. The objective of this field observation was to evaluate the efficacy of two different commercial *Coli-Clostridia* sow vaccines in reducing PWD-associated mortality in their off-spring.

Materials and Methods
On a commercial 425 head sow farm piglets were suffering from *E.coli* F4 PWD (weaning age 26 days). Control piglets (CP; n=1908) were born to sows vaccinated with a commercial *Coli-Clostridium* vaccine previously used on the farm and weaned between February and April 2017 (13 batches). Piglets born out of Entericolix-vaccinated sows (EP; n=2220) were weaned between May and June 2017 (12 batches). Date and reason of mortality (by judgement of the animal caretaker) from weaning to 45 days post-weaning were recorded per batch.

Results
Total mortality after weaning was lower for EP compared to CP (1.7% vs 2.2%). PWD-associated mortality was reduced significantly from 16 (0.8%) in CP to 3 (0.1%) in EP (OR 0.16; $P<0.005$). The average age of mortality due to PWD increased from 9 days (CP) to 29 days post-weaning (EP).

Conclusion
In this field observation mortality due to PWD after weaning was significantly reduced by the use of *Coli-Clostridia* combination vaccine compared to the previous farm protocol. Mortality was postponed to a later age in EP pigs, which might be explained by a longer lasting maternal immunity. With the expected ban off using high concentrations of zinc oxide in weaned piglet diets in the EU, alternative methods to protect against PWD gain further relevance. *Coli-Clostridia* sow vaccines can play an important role in this, specifically vaccines that provide long lasting protection.

Keywords: colibacillosis, vaccine, postweaning diarrhea, *E. coli*, maternal immunity

II -057

Comparative study to evaluate immunity induced by *E. Coli-clostridium* vaccines

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Introduction
Colostrum immunoglobins are a source of protection against microbial infections and confer passive immunity to the piglets until they have a mature immune system. The purpose of this study was to compare safety and efficacy of different *E. coli* vaccines by measuring specific antibodies against the main virulence factors in sows vaccinated with Porcilis®ColiClos or a competitor vaccine with the same indication (vaccine A).

Materials and Methods
In a Spanish farm (1,500 sows), 22 primiparous sows were randomly allocated in 2 groups. Prior to farrowing, sows were vaccinated with either Vaccine A (Ginseng adjuvant) or Porcilis®ColiClos, according to manufacturer's instructions. Three blood samples were collected from the sows prior to first and second vaccine dose and 2 weeks after 2nd dose and from piglets (54 piglets, 3 per litter). As a measure of vaccine efficacy antibody titers against specific *E. coli* antigens were measured with an ELISA test (internal MSD AH test). Safety of the vaccine was evaluated based on changes in body temperature and any adverse reactions and efficacy was based on antibody titers. Linear Method (GLM: program SPSS 15.0) was used for the statistical analysis.

Results
Safety: Feed intake was not impacted and no other adverse systemic or local reactions were observed. Efficacy: Antibody titers were higher in Porcilis®ColiClos than Vaccine A group: (987P: 9.67 vs 7.72 P=0.03; K88ab: 11.14 vs 9.75 P=0.03; K88ac: 10.65 vs 9.66 P=0.01; K99: 9.16 vs 7.34 P=0.11; LT: 8.23 vs 6.52 P<0.001).

Conclusion
Porcilis®ColiClos was safe and induced higher and more homogenous titers against every *E. coli* antigen than Vaccine A, which will result in better protection in piglets against *E. coli* enteritis. Achieving a high post-vaccination immunity in sows is important to ensure sufficient transfer of passive immunity to the current large litters.

Keywords: clostridium perfring, *Escherichia coli*, colostral immunity, piglet diarrhoea

VI-035

Effect of zinc oxide sources and doses on weaned piglets

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Introduction
Zn is commonly supplemented in piglets diets, at nutritional dosage (110 mg/kg Zn) to fulfil animal requirements or at pharmacological dosage (2400 mg/kg Zn) for its growth-promoting effect. High dosages of zinc oxide (ZnO) can improve performance through adjusting intestinal health, but Zn level in animal wastes may be high and lead to environmental concerns. Alternative solutions are consequently under investigation. In this study, a potentiated ZnO source (HiZox®, Animine), a coated ZnO and the standard ZnO were compared at different doses.

Materials and Methods
A total of 108 piglets, weaned at 21 days, were allocated to 18 pens (6 piglets/pen) and fed 6 experimental corn-soybean based diets during 14 days, with different zinc sources and doses: standard ZnO (110, NC; or 2400 mg/kg Zn, PC), coated ZnO (110 or 220 mg/kg Zn) or potentiated ZnO source (110 or 220 mg/kg Zn). Piglets were weighed individually at d 0 and d 14 and feed intake was recorded. At the end of the experiment, 3 piglets per pen were selected and sacrificed. Contents from proximal and distal small intestine were collected. Numbers of bacteria (*E. coli*, coliform bacteria) were assessed using selective media.

Results
There was no significant difference between the treatments for the growth performance. NC showed the lowest weight gain (1.2 kg) and PC the highest (1.6 kg). In the proximal small intestine, numbers of *E. coli* were significantly ($P< 0.05$) higher for NC and for the coated ZnO, compared to PC; the potentiated ZnO obtained intermediary results. In the distal small intestine, similarly, *E. coli* and coliform bacteria populations were significantly ($P< 0.05$) higher for the coated ZnO compared to PC.

Conclusion
Populations of *E. coli* and coliforms were significantly reduced by ZnO at pharmacological dosage, numerically decreased by the potentiated ZnO, whereas the coated ZnO showed no reductions. Complementary analyses are in progress.

Keywords: zinc oxide, piglet, intestinal health, bacteria, *E.coli*

VI-042

Influence of polyphenols (hydroxytyrosol) supplementation on oxidative and metabolic status of weaned piglets

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Introduction
Weaning is health-challenging for piglets, due to increased oxidative stress and pro-inflammatory status (which in turns increases oxidative stress). Current prophylactic strategies, after the use of antimicrobials for many years, aim to ameliorate the antioxidant/oxidative balance of the piglets, usually using antioxidant vitamins (specifically vitamin E, whose blood concentrations dramatically decrease at weaning). Alternative sources of antioxidants are polyphenols and, particularly, the current study aimed to analyze the usefulness of hydroxytyrosol. Hydroxytyrosol is an olive-derived polyphenol with prominent antioxidant activity, by itself and by increasing availability of vitamin E, but also with metabolism-regulatory properties.

Materials and Methods
Hence, a total of 153 weanling piglets (28 ± 1.9 days-old; 7.62 ± 0.29 kg) were used to compare the effects of supplementation with 50 ppm and 150 ppm of vitamin E (groups 50E and 150E, respectively) and 50 ppm of vitamin E plus hydroxytyrosol (MiaPhenol; 50 ppm; group HT). Blood samples were drawn weekly for 28 days after weaning to assess concentrations of hydroxytyrosol and vitamin E, lipid peroxidation (malondialdehyde; MDA), and parameters of glucidic metabolism (glucose, fructosamine) and lipid metabolism (triglycerides and total-, HDL- and LDL-cholesterol).

Results
Throughout the treatment, the group HT showed higher hydroxytyrosol concentrations than the other groups ($P<0.05$) and similar concentrations of vitamin E to the group 150E (higher in both groups than in the group 50E, $P<0.05$). There were no significant differences in plasma concentration of glucose, fructosamine and triglycerides among groups. However, total-, HDL- and LDL-cholesterol were higher in the group HT at one week after starting the treatment ($P<0.05$) while total- and LDL-cholesterol remained higher at the last sampling at 56 days-old piglets ($P<0.05$). In spite of such higher concentrations of lipids, the assessment of plasma MDA concentrations showed no differences in lipid peroxidation among groups.

Conclusion
In conclusion, the present trial indicates that the concurrent treatment with vitamin E and hydroxytyrosol favors the bioavailability of vitamin E and maintenance of high blood VitE-concentrations after weaning. Moreover, the addition of hydroxytyrosol may have a significant positive impact on cholesterol endogenous synthesis and availability, which is essential for normal growth and development in the early stages of life.

Keywords: piglets, vitamin E, Hydroxytyrosol, oxidative stress

VI-049

Sow colostrum and milk composition after supplementation of multi-strains probiotic Bactosac in feed during late gestation sow

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Introduction:
Bactosac is composed of multi-strains of lactic acid bacteria such as *Lactobacillus* spp., *Saccharomyces* spp. and *Pediococcus* spp. in liquid form. This product has been documented that can improve piglet growth and survival. Therefore, the present study was to further investigate the effect of probiotics supplementation during late pregnancy on colostrum composition.

Materials and Methods
At week 12th of pregnancy, 10 sows were selected and equally divided into 2 groups (control and treatment). Control-group was fed with normal feed while treatment-group was daily fed with normal feed plus 5 mL of Bactosac until farrowing. On day 113 of gestation, all sows were subjected to induce farrowing by injection of 5 mg Dinoprost into perivulva area. Colostrum samples in both groups were collected from all the teats as a pool sample (approximately 50 mL) at 3, 6, 12 and 24 hours (time at first piglet born was designated as 0 hour). All colostrum and milk samples were subjected to measure proportions of fat, protein, lactose, solid non-fat (SNF) and total solid (TS) by using MilkoScan™ Minor.

Results
Proportion of protein was highest after farrowing and dramatically declined at 24 hours, while proportions of fat and lactose were slightly increased by 1-2%. At 3 and 6 hours, all colostrum samples in control-group showed a slightly higher in all compositions than treatment-group, in contrast at 24 hours all colostrum composition in treatment- group showed higher some compositions such as fat (8.30±0.69 vs 6.41±2.25), protein (7.06±1.91 vs 6.10±2.48) and TS (20.59±1.35 vs 17.26±5.33) than in control-group. Considering the differences in colostrum composition between groups, sow fed with Bactosac during late gestation produced more proportions of fat (+1.89), protein (+0.96), lactose (+0.35), SNF (+1.08) and TS (+3.33) during the first 24 hours of lactation. This differences may responsible for increase body weight gain of piglet during 1-2 days old and subsequently increasing weaning weight.

Conclusion
In conclusion, supplementation of Bactosac (5 mL/sow/day) by top dressing from 4 weeks before farrowing has beneficial effect on the colostrum and milk composition during the first 24 hours after farrowing.

Keywords: sow colostrum, probiotics, *Lactobacillus* spp., *Saccharomyces* spp., *Pediococcus* spp.

VI-050

Supplementation with organic acids showing different effect on growth performance, gut morphology and microbiota of weaned pigs fed with highly or less digestible diets

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Introduction

Two studies were conducted to evaluate effects of organic acids (OA) in a highly digestible (Exp. 1) or less digestible diet (Exp. 2) on growth performance and intestinal health of weaned pigs.

Materials and Methods

In Exp. 1, a total of 240 pigs weaned at d 21 were assigned to one of five dietary treatments, NC (basal diet, 3000ppm ZnO in first 2 weeks only), PC (NC+10 mg/kg zinc bacitracin, 5 mg/kg colistin sulphate and 5 mg/kg olaquindox), OA1 (NC+0.2% blend of encapsulated butyrate, MCFAs, organic acids, and phenolics), OA2 (NC+0.3% blend of free and buffered short chain fatty acids combined with medium chain fatty acids) and OA1+OA2 (NC+0.2% OA1+0.3% OA2) for 49 days. All treatments in Exp. 1 used the same highly digestible basal diet. At d 28, 8 pigs from each group were sacrificed to collect intestinal and digesta samples for biochemical analysis.

Results

The growth performance and intestinal morphology were not affected by the treatments. However, piglets with OA2 had a lower number of *Escherichia coli* ($P< 0.05$) in colon. In addition, OA1, OA2 or their combination resulted in higher concentrations of acetate and propionic acid in cecum and colon ($P< 0.01$) compared with NC. A less digestible diet without high ZnO was used in Exp. 2. A similar design was used, except that OA2 was replaced with another organic acid blend (OA3, a blend of free and buffered organic acids). Compared with the NC, supplementation with OA1 and OA3 in low digestible diet improved ADG and F: G in the seventh week post-weaning ($P< 0.01$), decreased the diarrhea index of pigs during the first three weeks post-weaning ($P< 0.05$), increased ileal villus height ($P< 0.05$), and propionic acid concentrations in colon content ($P< 0.05$) as well. Moreover, genus *Prevotella* in colon was increased and microbiota community structures was significantly different in the OA1+OA3 treatment.

Conclusion

The present research indicated that dietary supplementation with OAs improved intestinal health. The organic acid blends showed a similar growth-promoting effect as antibiotics in the less digestible diet where no high ZnO was added.

Keywords: organic acids, intestinal morphology, weaned pigs, microbiota composition

VI-009

Sows with prolonged parturition already compromised from the start of expulsion

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Introduction
This study aimed to determine factors that contribute to asphyxia and stillbirth, and to establish consequences of varying degree of asphyxia for neonatal performance of piglets.

Materials and Methods
Multiparous Large White x Landrace sows (Hypor, Hendrix Genetics, n = 47) were monitored continuously around parturition to record time of birth and condition of each piglet, collect mixed cord blood samples, colostrum intake based on piglet weight at birth and 24 h later, and performance to weaning (n = 515) and 10 wks of life (n = 302). Degree of asphyxia was defined by umbilical blood lactate : <3.36, 3.36 to 4.45, 4.46 to 6.40, or >6.40 mmol/L.

Results
Sows had 15.3 ± 0.5 total born piglets on average. Time to give birth to the first four piglets was strongly predictive (r=0.74) of total duration of expulsion. Sows that took >300 min to farrow had 1.6 ± 0.4 stillborn piglets, compared to 1.0 ± 0.4 stillborn in sows with average duration of farrowing, and 0.5 ± 0.2 stillborn in sows that took short (<200 min) to farrow (P < 0.10). Blood lactate in umbilical cord samples increased linearly from 4.23±0.16 mmol/L for the first three piglets, to 6.34±0.30 mmol/L in piglet 13 and over. Similarly, the risk of stillbirth increased from 2% in the first three piglets to 19% in piglet 13 and over. Birth order and blood lactate also affected pre-weaning survival. Piglets with <4.5 mmol/L lactate had a 5% risk of neonatal mortality compared to 10% for piglets with >4.5 mmol/L. Piglets with highest degree of asphyxia had 22% lower birth weight (P<0.01), took 6 min longer from birth to first milk intake (P<0.01), had 22% less colostrum intake (P=0.10), gained 6% less to weaning (P<0.05), and gained 9% less to 10 wks of life (P<0.05).

Conclusion
Duration of parturition and birth order clearly affect asphyxia and risk of stillbirth, as well as neonatal performance. At onset of expulsion, duration of parturition is determined already.

Keywords: parturition, stillbirth, asphyxia

VI-022

Using lung scoring as a tool to evaluate thoracic lesions in pigs from selected slaughterhouses in the province of Batangas, Philippines

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Introduction
Respiratory disease is one of the major causes of mortalities of pigs in the Philippines and can lead to poor productivity and economic losses. Lung scoring is a widely accepted technique to evaluate thoracic lesions and can be used for respiratory disease surveillance in swine. Four veterinarians from Batangas (Region IV-A, Philippines) were trained in lung scoring prior to conducting a three-month study in the province from October to December 2016. The study aimed to (i) determine the prevalence of thoracic lesions caused by respiratory diseases in swine at slaughterhouses, and (ii) establish baseline lung score data for the province.

Materials and Methods
A total of 260 pigs from five slaughterhouses were included in the study. Lungs were scored for cranio-ventral pneumonia using a scale of 0 to 55 and for pleurisy from 0 to 3. Presence or absence of pericarditis was recorded.

Results
The median lung score was 7, interquartile range 2-19, and range 0-53. Using a threshold of ≥7, 135 pigs (51.9%) had a high lung score, 148 pigs (56.9%) had a pleurisy score of at least one and 64 (24.6%) had pericarditis. Co-occurrence of thoracic lesions was common: 43 (16.5%) pigs had a high lung score, pleurisy and pericarditis, 77 (29.6%) had two of these lesions, 64 (24.6%) had one and 76 (29.2%) had none of these lesions. Of the 225 pigs with a non-zero lung score, 187 (83.1%) showed acute cranio-ventral pneumonia lesions with remainder showing chronic lesions. Pigs from commercial farms were more likely to have high lung scores, pleurisy and pericarditis compared to pigs from backyard farms.

Conclusion
This study has provided baseline lung score data for pigs in Batangas. Subsequent monitoring will enable assessment of changes in respiratory disease frequency in the province.
This work is supported by ACIAR and PCAARRD.

Keywords: lung scoring, respiratory disease, Philippines

VI-032

Replacing in-feed antibiotics with feed additives and the effects in performance and immune response of weaning piglets

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Introduction

Exposed to infectious agents or their molecular patterns, immune cells react and the immune response (IR) can affect growth performance (GP). Haematological outcomes, such as red blood cell counts (RBC), white blood cell counts (WBC), and immunological such as total serum immunoglobulin G (IgG) and total antioxidant capacity (T-AOC) could vary. This study investigates the effect of replacing in-feed antibiotics with feed additives (FA) based on short and medium chain organic acid blends (SCFA, MCFA; OA) on GP and IR of weaning piglets (WP).

Materials and Methods

28 days old WP (n=144) with equal sex ratio and starting weight (8.09±0.11 kg) were randomly assigned to treatment groups: negative control (NC), positive control (aureomycin 75 ppm) (PC), Treatment A (OA) (TA), and Treatment B (OA) (TB), with 6 replicates of 6 piglets. Average daily gain (ADG) and feed conversion (FCR) were calculated over the 28 d period. On d 14 and d 28, 2 piglets of each replicate were randomly selected for blood sampling, complete blood count and immune-related measurements were analyzed. On d 24, fresh fecal samples in each replicate were collected for 16S rRNA sequencing and bacterial diversity analysis. The data was analyzed by ANOVA using the general linear models (GLM) procedure of SAS 8.0.

Results

Piglets in TB had higher ADG (NC 164.25 vs TB 195.84) ($P=0.02$), and lower FCR (NC 2.32 vs TB 2.09) ($P<0.05$) in relation to NC. Compared to NC, TA and TB increased RBC ($P<0.01$), WBC, and eosinophil percentage on d 28, and increased IgG and T-AOC level on d 14 and T-AOC level on d 28 ($P<0.05$). TA and TB increased *Ruminococcaceae*, *Lachnospiraceae*, *Eubacterium coprostanoligenes* and *Lactobacillus* levels. *Faecalibacterium* in TB was higher compared to PC ($P<0.05$).

Conclusion

The results indicate that diets supplemented with OA could improve GP by increasing proliferation of beneficial bacteria in the digestive tract and possibly by modulating the IR of WP. The modulation of the innate IR is expressed by increasing cells associated with both pro-inflammatory and anti-inflammatory messages and pathways, increasing the humoral component of the adaptive IR and increasing anti-oxidation capacity. Further research is necessary to define the mechanism of action.

Keywords: antibiotic reduction, feed additives, organic acids, MCFA, immunology

VI-053

Assessing differences in microbiome between cull, sick, and healthy sows

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Introduction

Culling is an essential and common practice in swine herds in order to keep high productivity and profitability. Most times, the definitive underlying reason for culling is not completely elucidated. The microbiome is an emerging area of study for food animals such as pigs; and it has been reported that both gut and nasal microbiome are associated with susceptibility to respiratory conditions such as PRRS, PCV2, and Glasser's. However, information regarding the microbiome in the cull sow population is lacking.

Materials and Methods

The objective of this study was to investigate whether there was a difference in the diversity of bacterial microbes in the gut and respiratory tract of sows of different health status. A cross-sectional study was conducted during Summer 2017 in six sow farms from the same production system. Farms were visited once, and 60 samples were collected: 30 nasal swabs, and 30 fecal samples, from three different categories of animals: healthy (10), cull (10), and sick sows (10). Samples were transported to the laboratory in dry ice and pooled (1:5) to equal volume prior to DNA extraction. Microbiota analyses were performed after sequencing the 16S rDNA V4-V5 variable region using MiSeq. Reads were compared to the GreenGene database (08-2013 release) using QIIME.

Results

Results revealed that fecal samples showed significantly higher operational taxonomic units (OTU) richness compared to the nasal samples. Within the phyla found in nasal cavities and fecal samples of sows, *Proteobacteria* and *Firmicutes* were the most abundant, respectively. Interestingly, we observed an important farm-level diversity difference in the fecal microbiota; however, this was less obvious for the nasal microbiota. Finally, samples obtained from cull sows yielded more diverse nasal microbiota and was similar to sick sows than to healthy sows; however, they showed less diverse gut microbiota as compared to either healthy or sick sows.

Conclusion

In conclusion, there was a difference in the diversity of bacterial composition in the gut and respiratory tract of sows of different health status. Future study goals include testing differences in abundance of specific bacterial communities between groups of animals, as well as assessing farm-level differences that may explain the observed findings.

Keywords: cull sows, microbiome, metagenomics

IV-058

Does the supplementation of natural progesterone or altrenogest have any effect on sows' corpora lutea development? (preliminary results)

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Introduction
Variability in progesterone concentration during early pregnancy can affect the secretion of nutrients, cytokines and growth factors on the histotroph as its influence endometrial development. Gilts supplemented with exogenous progesterone exhibits increased total protein content in the uterine lumen and augmented endometrial expression of genes responsible for successful pregnancy establishment and placentation. However, high doses of exogenous progesterone, for long periods, can impair normal development of the corpus luteum (CL) resulting in decreased progesterone production throughout gestation. The objective of the present study was to evaluate whether progesterone supplementation during early pregnancy affects CL development of sows.

Materials and Methods
Sixteen sows from a total of sixty with parity ranging from 2 to 5 were blocked by percentage of body weight loss after 21 days of lactation period. The moment of ovulation was detected by transcutaneous ultrasonography. Sows were inseminated in the oestrus following weaning and allocated into one of three treatments: without hormonal supplementation (CON), supplemented with 2.15 mg/kg/IM of long-acting natural progesterone on day 6 after ovulation (PG) and supplemented with altrenogest from day 6 to 12 after ovulation (RU). Sows were slaughtered on days 13 (n=8) and 28 (n=8) of gestation. All CL's were collected from the ovaries, individually weighted and their volume was calculated.

Results
There was a difference for CL's development on both 13 and 28 days of gestation ($P<0.05$); at 13 days of gestation CON and RU had similar values for weight and volume, and PG had similar volume when compared with CON and higher volume when compared with RU. Finally, PG had a higher weight than RU and CON. At 28 days of gestation, PG had a higher weight than CON and similar to RU while there was no difference among CON and RU ($P>0.05$). Regarding CL's volume, RU had higher values than CON, and similar to PG, while PG and CON were similar.

Conclusion
Based on weight and volume as parameters for CL's development, obtained to the present date, hormonal treatment with progesterone or progestin seems to be beneficial on both 13 and 28 days of gestation.
Acknowledgements FAPESP Grant 2017/00290-0

Keywords: progesterone, altrenogest, corpora lutea, pregnancy

Ⅳ-047

High aerial ammonia as cause for increased rate of return to oestrus in sows; a case report

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Introduction
Non-infectious causes of reproductive failure are most often detected when farms report an increased rate of return to oestrus (RTO) with a regular interval between inseminations. However, non-infectious causes can result in RTO with an irregular interval between inseminations due to early embryonic death (EED) because of chronic physical stress.

Methods
This case report considers the data analysis, farm inspection and evaluation of feed, feeding, behaviour, climate and housing with regard to a farm of 500 sows experiencing an increased rate of return to oestrus.

Results
Data analysis an increased rate of RTO (average 11%) in sows of all parity. In addition an increased rate of too small litters was found. Finally, in 2nd cycle sows, a classical second litter syndrome was observed . The data, nor the anamnesis revealed any increased rates of abortions, mummies, decreased gestation length or diseased sows thereby excluding infectious causes of RTO and thereby suggesting EED. An evaluation of stressors for gestating sows was needed to detect the cause of EED. A thorough evaluation of feed and feeding (in electronic sow feeding stations), as well as human-sow interaction did not reveal any abnormalities. However, video behaviour analysis revealed that ~75% of gestating sows rest in a sternal position, when instead lying in flank position is considered to be normal. Analysis of the climate analysis revealed a slightly increased ambient temperature (25℃) as well as an elevated aerial ammonia concentration (75 - 112 ppm) due to the fact that incoming air fell into the manure pit. Analysis of the manure pit construction and housing revealed an improper design to fit housing of gestating sows.

Conclusion
As no other cause of EED was found it was concluded that the high aerial ammonia is the likely cause of EED on this farm. Advice is currently being implemented to reduce ambient temperature and pit ventilation. However, improper housing design restricts this farmer to implement a scientific based and sustainable solution. This case shows that combining extensive data analysis and a thorough evaluation of stressors enables the identification of causes of EED on farms.

Keywords: return to oestrus, chronic stress, ammonia, reproduction

IV-043

Prevalence of fecal pathogens in different swine production phases

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Introduction
Enteric disease is common in pig herds and causes major losses to the pig industry (production chain). *Escherichia (E.) coli* F4, F5, F6 and F41, *Clostridium (C.) difficile* and *perfringens*, protozoa and *Rotavirus* are important causes of diarrhea in suckling pigs, whereas *E.coli* F4 andF18 and *Rotavirus* are important in weaned pigs. Animals in other age groups may also carry these pathogens and be important for within-herd transmission (infection chain). The present study assessed the prevalence of shedding of these pathogens by pigs in different age groups in commercial pig herds (infection pressure).

Materials and Methods
Fourteen European pig herds without specific herd problems were selected. Within each herd, rectal feces samples were taken from randomly selected sows, weaned pigs and fattening pigs (15-20 animals per herd). A rapid immunochromatography diagnostic test (Rainbow Piglet, Bio-X diagnostics s.p.r.l.) was used to determine the presence or absence of eight pathogens or their virulence factors: *E.coli* F4, F5, F18 and F41, *Cryptosporidium*, *Rotavirus* and *C. difficile* and *C. perfringens*. The prevalence of each pathogen was calculated by dividing the number of positive animals by the total number of tested animals in each specific age group.

Results
The most prevalent pathogens in sows were *C. perfringens* (60%), *Cryptosporidium* (19%) and *E.coli* F5 (12%). Fattening pigs showed the highest prevalence of *C. perfringens* (69%), *E.coli* F5 (21%) and *Cryptosporidium* (21%). *E. coli* F4, F18 and *C. difficile* were incidentally present in sows and finishers. Besides *E. coli* F4 (11%), *E. coli* F41 (18%), *C. perfringens* (73%) and *C. difficile* (21%) were found in weaned pigs. If *E.coli* F5 was present on a farm, it was present in all production stages in 46% of the farms. Similar data were found for *E.coli* F41 (33%), *C. perfringens* (43%) and *Cryptosporidium* (42%).

Conclusion
Although causal relationships cannot be established with the present study design, the results suggest weaners to serve as a potential source of *C. perfringens*, *E. coli* F41 and *C. difficile*, while sows and grower/finishers may serve as a source of infection of *C. perfringens*, *E. coli* F5 and *Cryptosporidium*.

Keywords: infection pressure, production chain, infection chain, enteric disease, cryptosporidium

IV-070

Evaluation of non-infectious causes of pre-weaning mortality in piglets

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Introduction
Piglet pre-weaning mortality (PWM) is one of the major issue that affects herd productivity in the swine industry. Knowledge of factors that influence piglet PWM are important to reduce production loss, improve animal welfare and to raise profits opportunities in commercial herds. The objective of this study was to identify the risk factors for non-infectious PWM in a commercial farm of 4k sows (PIC Camborough®).

Materials and Methods
A total of 10,959 piglets from 805 farrowing were monitored and all dead piglets (1,600) were necropsied to identify the cause of death. The variables evaluated were: age, cause of death, presence of colostrum/milk in the stomach, loss of body temperature, feeding of sow and execution of routine managements, such as: cross fostering, handling sows at farrowing and split suckling. The data were analyzed using the SAS (Statistical Analysis System, version 12.1), were analyzed using the Kruskal-Wallis test for non-parametric data.

Results
Overall, the PWM rate was 14.6%. The main cause of death was crushing (72%) followed by elimination (25.3%) (P<0.0001). Piglets deaths occur mainly during the first 72 h after farrowing (52.6%) where 67% of the dead piglets had no stomach contents (P<0.0001). The quality of cross fostering, handling sows at farrowing and split suckling managements practices were not in place (P<0.005) and strongly contributed to PWM. In the first, second and third week of lactation 19.2%, 28.9% and 36.5% of sow feeders had no feed (P<0.0001). The evaluation of body temperature loss showed that rooms with thermal mat, light and wind break contribute with an increase in body temperature of newborn piglets (+0.5°C) 2 h after birth (P<0.005), however low weight piglets (<800 g) had greater thermal variation (6.05°C; P<0.005). The number of deliveries/employee attended at the same time was 8.3 and the daily labor availability was 24 minutes (or 5.7%) per weaned piglet.

Conclusion
Based on our results, several steps can be recommended to reduce PWM rate: Insure that farm staff are focused on prioritizing piglet survival, drying newborn piglets whenever some need special attention, conducting split-suckling, monitoring the litter until weaning and promote good management and nutrition pre- and post-farrowing can increase piglet survival.

Keywords: management, colostrum, piglet survival, newborn, body temperature

IV-030

Elimination of Senecavirus A from a breeding stock flow

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Introduction
The inability to distinguish Senecavirus A (SVA) lesions from reportable vesicular diseases emphasizes the significance of SVA. When SVA appears in a multiplier population, steps must be taken to prevent the spread within and between flows and downstream to customers. This report describes the steps taken to eliminate the virus from a seedstock breeding herd and associated growing population.

Materials and Methods
When a breed-to-wean multiplier herd became infected with SVA, breeding stock sales from the sow herd and its two off-site gilt finishers were immediately halted. Aggressive efforts exposed the resident sow herd and its on-site quarantine facility to the virus. Two off-site wean-finish facilities (wffs) receive breeding gilt candidates operating as continuous flow. Weanings were diverted away from these sites, but vesicles eventually appeared at both wffs. SVA elimination was initiated, building on the AASV sampling/testing model for PRRSV elimination. Negative status was established over time based on the absence of lesions in the sow herd, negative oral fluid PCRs from weaning-aged pigs, introduction of naïve sentinels with no clinical signs of SVA and negative sentinel serum PCR testing 4-6 weeks after exposure. Conditionally-negative pigs were weaned into depopulated, washed, disinfected and dry wffs. Close observation for SVA lesions and PCR oral fluid testing continued in the finisher populations.

Results
No downstream herds were exposed to SVA through delivery of breeding stock. Sentinels entered the sow herd nine weeks after exposure. With favorable sentinel results, weaning into wff resumed 13 weeks post-exposure. Sentinels and subsequent wff flows never showed clinical signs and were SVA PCR negative.

Conclusion
Prompt aggressive action restricted SVA to the multiplier flow. SVA was eliminated from the sow herd within 13 weeks of first clinical signs. Depopulation, thorough cleaning and downtime removed SVA from the wffs.

Keywords: Senecavirus, elimination

V-026

An integrated management-based approach for surveillance and control of zoonoses in emerging livestock systems: Myanmar Pig Partnership (MPP)

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Introduction

The project focuses on the pig production and processing sectors in Yangon region, Myanmar which, of all the countries in the world, is expected to show the most rapid growth in pig production to 2030. We are applying interdisciplinary expertise including social and biological sciences with state veterinary services to characterise changing pig production systems and associated bacterial pathogens, the social and economic drivers of AMU on farms and the landscape of veterinary healthcare.

Materials and Methods

A year-long baseline longitudinal veterinary survey of 18 farms and 10 slaughterhouses, through the winter, summer and rainy seasons, and comprising a total of more than 1200 faeces, tonsil, environmental and slaughter samples was concluded. It revealed a high prevalence of non typhoidal *Salmonella* (NTS) and *Streptococcus suis* – both important zoonotic pathogens of pigs- including seasonal differences in environmental contamination by NTS. Isolates are undergoing antimicrobial susceptibility testing and whole genome sequence analysis. Collated on-farm productivity and health information will be integrated with data emerging from the social sciences-based aspects of our project.

Results

The in-depth ethnographic observation revealed diseases are understood by farmers, where they seek help for illness in pigs, what practices are followed when disease outbreaks occur in a village, and how farmers see their interaction with animals and pigs in particular.

The value chain mapping methodology revealed an important difference between pork consumed in rural and urban areas. The supply chain is longer and more complex in the case of urban markets than rural markets. It also points at the nuanced understanding of food safety and traceability by consumers. The combination of these two methods reveals insights into the broader structural context of different scales of pig farming and how this influences decision-making by farmers with respect to treating and marketing of their pigs, and the use of antimicrobials.

Conclusion

We will integrate this data to identify opportunities for culturally relevant and livelihood relevant training to support increased productivity, food safety and animal health.

Keywords: zoonosis, surveillance, emerging livestock systems

V-002

Instestinal lymphangectasis and lipogranulomatous lymphangitis in pigs

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Introduction
The objective of this study was to describe cases of intestinal lymphangectasis and lipogranulomatous lymphangitis (ILLL), comprising 4 affected pigs. ILLL is a protein-losing enteropathy, frequently associated with lymphangiectasia. ILLL is rare in pigs, usually an accidental finding in the slaughter house. ILLL can be confounded with other intestinal lesions, such as lesions caused by *Mycobacterium avium* ssp. paratuberculosis, and therefore, must be considered in the differential diagnosis.

Materials and Methods
Two cases were included in the investigation. Case 1 comprised a 19 weeks-old pig. Formalin-fixed and non-fixed tissues were collect from case 1. Case 2 comprised formalin-fixed intestinal samples from 3 slaughtered pigs. Gross lesions were described by the veterinarian practitioner as gas filled "bubbles" in the serosa of the small intestine and surrounding tissue. Samples from case 1 were tested for differential diagnosis: bacteriology, acid fast stains (*Mycobacterium* sp.), molecular diagnosis (PRRSv, parvovirus, circovirus type 2 - PCV2) and immunohistochemistry (PVC2). The cases were analyzed by histopathology (HE).

Results
Laboratory testing. Beta-Hemolytic *Streptococcus* sp. was isolated in aerobic culture and interpreted as post-mortem contaminant. Intestinal samples were PCR positive for porcine parvovirus 2 and negative for PRRSV, porcine circovirus type 2. Sections of intestine were negative for acid fast bacilli and for porcine circovirus type 2 by immunohistochemistry. Histopathology. Small intestine showed dilated lymphatics, diffusely distributed throughout the submucosa and tunica serosa. Cystic lymphatics were surrounded by collagen tissue showing fibroplasia and lymphohistiocytic inflammatory infiltrated, with intralesional multinucleated giant cells and epithelioid macrophages.

Conclusion
The present study represents one of the scarce reports of ILLL in pigs. Obstruction or any other cause of increased intraluminal pressure in the lymphatic vessels can result in leakage of lymph fluid to the interstitium, leading the histiocytic inflammation. ILLL does not interfere with the acceptability of the carcass. Association with *Clostridium* sp. and coliforms is hypothesized, although not confirmed. Although not a concern for food safety, ILLL is an important differential diagnosis for Johne's disease, which, though rare in pigs, is important in other animal species and is a potential zoonotic disease.

Keywords: protein-loosing ente, lipogranuloma, granulomatous enteri, chronic enteritis

V-012

Dynamics of Extended-spectrum β-lactamase (ESBL)/AmpC-producing *Escherichia Coli* levels in the intestinal tract of weaned piglets

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Introduction
According to the World Health Organization, antimicrobial resistance (AMR) has reached alarming levels in both humans and animals. Extended-spectrum β-lactamase/AmpC-producing *Escherichia coli* (ESBL/AmpC) are a frequently detected group of AMR bacteria. In this study the effect of weaning, pathogenic *E. coli* challenge and different feed interventions on the presence of ESBL/AmpC in the intestinal tract of piglets was determined.

Materials and Methods
Forty naturally ESBL/AmpC infected piglets were individually housed after weaning. Piglets were fed either a control diet; a diet containing a blend of formic and lactic acid at 2 kg/tonne (FL); a diet containing FL and 2 kg/tonne rye overgrown with mycelium of Agaricus subrufescens (FL-ROM); or a diet containing FL, 1 kg/tonne ROM, and 1 kg/tonne mannanase hydrolyzed copra meal (FL-ROM-CM). All piglets were infected with enterotoxigenic *E. coli* F4 (ETEC) at five days after weaning. At day 5 and 12 after weaning presence of ETEC was determined by qPCR. At day 0, 5, and 12 after weaning ESBL/AmpC in feces of piglets were enumerated by overnight incubation of serial diluted fecal samples on cefotaxim containing selective MacConkey agar plates.

Results
The prevalence of ETEC was 0% at day 5 and increased in all treatment groups to 70-100% at day 12. ESBL/AmpC-levels in feces were equal for the different treatment groups at day 0 (2.8 log CFU/g) and day 5 (2.0 log CFU/g). At day 12 the level of ESBL/AmpC increased (P<0.05) to 4.9 log CFU/gram feces in the control group; but not in the FL-group (3.8 log CFU/g) or the FL-ROM group (3.0 log CFU/g) and was lower in the FL-ROM-CM group (1.3 log CFU/g) compared to the control group (P<0.05).

Conclusion
In conclusion, weaning did not affect ESBL/AmpC shedding; however, ESBL/AmpC shedding increased after ETEC infection, possibly due to transfer of resistance genes from resident ESBL/AmpC to the enterotoxigenic strain or overgrowth of resident ESBL/AmpC after *ETEC* infection. The FL-ROM-CM combination prevented this increase; and therefore may be useful in reducing ESBL/AmpC in weaned piglets.

Keywords: *Escherichia coli*, ESBL/AmpC, piglets, feed additives

V-014

Tailor-made coaching antimicrobial reduction in pig farms within the Belgium – Dutch cross border project; i-4-1-health

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Introduction

Antimicrobial use in pigs (AMU) has led to an increase in antimicrobial resistance (AMR). This has prompted measures to reduce AMU, which has been associated with AMR reduction on national level. However, it remains unclear how on-farm dynamics of AMU and its effects on AMR are exactly related. Moreover, it is challenging to influence farmers' behavior towards increased infection prevention and AMU reduction. In this project we use specific coaching skills to reduce AMU and in addition evaluate the effects on AMR.

Materials and Methods

The i-4-1-health project started in 2017. In Flanders as well as in The Netherlands, 15 pig farms with an above average AMU are visited four times in 1.5 year. During the first visit, an assessment is made of e.g. farm biosecurity, technical performance, AMU and AMR. AMR is determined in faecal samples phenotypically in *Enterobacteriaceae*. Genotypical and resistome analyses will be performed later. The results of the assessment are evaluated using a designated new tool (V-iris) to start coaching four weeks later. In the coaching process, farmers and veterinarians reflect upon their own perceived behavior. A tailor-made action plan will be developed together with the farmer and herd veterinarian. After 6 and 12 months the farm is revisited to evaluate implementation and motivate the farmer for the continuation of the action plan. At the 2nd and 3rd visit faecal samples are obtained for analysis of AMR development.

Results

The farm visits started recently and preliminary results will be presented.

Conclusion

In both countries veterinarians and farmers face the challenge to reduce AMR. Preliminary experiences indicate that there is not a one-size-fits-all approach within, nor between countries. To increase knowledge and awareness during the project, knowledge sharing sessions are organized for people working in public health, human medicine and veterinary medicine.

Keywords: coaching, antimicrobial use, one health, resistance, AM reduction

V-006

The use of organic acids at lairage and its effect on pig *Salmonella* shedding at slaughter

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Introduction
The presence of *Salmonella* in the pig's feces is a major source of abattoir and carcass contamination. The main objective of this study was to assess whether the addition of organic acids to the water of abattoir pens (during lairage) may be a useful strategy to reduce the proportion of pigs shedding *Salmonella*, therefore mitigating the risk of *Salmonella* slaughter contamination.

Materials and Methods
The study was carried out during 2017. Pigs coming from the same farm and in the same truck, were unloaded into an abattoir pen with the treated water (40 pigs - treated group) and pens with regular water (rest of the pigs - control group). After a waiting period of 10-14 hours, pigs were slaughtered and after evisceration fecal samples collected to determine the presence of *Salmonella* (ISO 6579: 2002/Amd 1:2007). The prevalence of shedding was compared between both groups by Fisher's exact test. A type of formic acid esterified in the form of glycerol (MOLI-M C1, Molimen SL, Barcelona, Spain) was used as treatment (10 kg/1000L of water). Five replicates of this field trial were carried out for this study.

Results
Overall, in 115 (57.5%) out of 200 pigs in the control group *Salmonella* was found in feces, compared to 81 (40.1%) out of 202 in the treated group ($P<0.01$). Since the effect may be also related to the overall amount of water consumed, analysis was repeated after grouping field trials into two categories: water consumption between 0.5-1 litre/head, and >1 litre/head. After adjusting by this variable, results remained similar. The Mantel-Haenszel adjusted odds of shedding *Salmonella* was higher (OR = 2.2; 95%CI = 1.5-3.4) for the control group.

Conclusion
In conclusion, these preliminary results suggest that the addition of this type of organic acid in the water of slaughter pens could help to decrease somewhat the proportion of pigs shedding *Salmonella*. More field trials using different doses or amount of water consumption should be carried out to assess the real potential of this approach.

Keywords: lairage, organic acids, *Salmonella*, slaughter, water treatment

V-015

The prevalence and load of *Salmonella*, and key risk points of *Salmonella* contamination in a swine slaughterhouse in Jiangsu province, China

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Introduction
We investigated *Salmonella* contamination in a swine slaughterhouse in Jiangsu province (China) by analysing prevalences, loads, and serotypes of *Salmonella* isolates.

Materials and Methods
In total, 480 samples were collected, with total prevalence of 25.4%.

Results
High *Salmonella* prevalence and load were observed at exsanguination and splitting stages (40.6% and 75.0%; 2.50±0.94 and 2.24±0.72 log MPN/m^2, respectively), with low prevalence and load at dehairing, flaming, and chilling stages (2.5%, 5.0%, and 15.0%; 1.39±0.42, 1.36±0.31, and 1.38±0.30 log MPN/m^2, respectively). The *Salmonella* prevalence and load increased substantially through polishing, rectal drilling evisceration and splitting stage, then basically decreased through following stages. Six serovars were represented among 122 *Salmonella* isolates; *S. Derby* and *S. Typhimurium* were predominant and were subtyped using PFGE and CRISPR typing approaches. Both the typing approaches indicated the *Salmonella* from same sampling visit, *Salmonella* isolated after splitting, and *Salmonella* isolated from splitter and carcass swab-samples were highly resemble.

Conclusion
Our findings revealed that four slaughtering stages (polishing, rectal drilling, evisceration and splitting) were important risk points for *Salmonella* release; introduced *Salmonella* was the major source of swine carcass contamination. Post-splitting slaughtering processes were major contamination risk points, and the splitter was a contamination factor. Our data suggest routes for controlling *Salmonella* contamination in a swine slaughterhouse.

Keywords: *Salmonella*, swine slaughterhouse, PFGE subtyping, CRISPR subtyping, contamination

V-019

High prevalence and multidrug resistance of salmonella from retail pork in Guangdong, China

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Introduction
The aimed to investigate prevalence, serotype distribution, antimicrobial resistance (AMR) and to characterize extended spectrum β-lactamases (ESBLs) producing *Salmonella* isolates from retail pork in Guangdong province, China.

Materials and Methods
Samples were collected during one year period (March 2016—February 2017) on monthly basis obtained from six cities (including Guangzhou, Shenzhen, Heyuan, Shaoguan, Foshan and Yunfu; Choose 3 retail markets in each city; pork types (i.e., dressed porks) and seasons (i.e., spring, summer, autumn and winter)) in Guangdong province, China. A total of 428 retail pork samples were purchased.

Results
In which, high levels of *Salmonella* contamination was detected in pork (313/428, 73.1%). Twenty-five serotypes were identified in 313 *Salmonella*, and *Typhimurium* (78/313, 24.9%), *Rissen* (67/313, 24.1%), *Derby* (66/313, 21.1%) and *London* (48, 15.3%) was the most dominant serotypes in retail porks. High antibiotic resistance rates were found for sulfisoxazole (256/313, 81.8%), tetracycline (247/313, 78.9%) and ampicillin (199/313, 63.6%). Notably, found a small minority of the resistant rate of cephalosporins, polymyxins and carbapenes such as cefotaxime (10/313, 3.2%), ceftazidime (4/313, 1.3%), cefepime (9/313, 2.9%), polymyxin B (5/313, 1.6%) and imipenem (3/313, 1.0%). Multidrug-resistance (MDR) was detected in porks (229/313, 73.2%). Resistant rate of *Salmonella* in different serotypes vary widely. Especially, isolates such as *S. Typhimurium* and *S. Rissen* exhibited highly resistance to antimicrobials. The MDR of *Typhimurium* and *Rissen* is significantly higher than that of *Derby* and *London* ($P<0.05$). Seven *Salmonella* isolates were identified as ESBLs-producing, covered 2 *Salmonella* serotypes (including *Typhimurium* and *Derby*) and displayed different PFGE genotypes. $Bla_{CTX\text{-}M\text{-}55}$ was the common ESBLs gene (5/7, 71.4%), followed by $bla_{TEM\text{-}206}$ *and* $bla_{TEM\text{-}214}$ (2/7, 28.6%).

Conclusion
This study indicated that *Salmonella* was widespread in retail pork in Guangdong province and has high multidrug-resistance, especially multidrug-resistant serotype *Salmonella*. So, suggests that should focus on *Salmonella* serotype and strengthend the long-term monitoring of MDR serotype *Salmonella* in retail animal-derived foods.

Keywords: *Salmonella*, retail pork, prevalence, antimicrobial resistance

V-011

Enhanced immune response during *Salmonella* infection improves animal health and performance in weaned piglets

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Introduction
According to the World Health Organization, antimicrobial resistance has reached alarming levels resulting in an urgent need for new antibacterial strategies. Several feed additives can enforce gut health or more specifically kill and bind intestinal pathogens and enhance immune response. However, the effect of immune modulation during infection on animal health and performance is unclear.

Materials and Methods
Twenty-four individually housed weaned piglets consumed either a control diet or the same diet supplemented with formic and lactic acid for antibacterial properties and mannanase hydrolyzed copra meal and rye overgrown with mycelium of Agaricus subrufescens for gram-negative bacteria binding and immunomodulatory properties. Piglets received a feed matrix containing 109 CFU Salmonella typhimurium from 5 days after weaning for 7 consecutive days. *Salmonella* and ESBL/AmpC-producing *E. coli* (ESBL/AmpC) fecal shedding was determined 1-4 days post infection (dpi). Furthermore, Il-1β, Il-6, Il-8, Il-12, IgA, and calprotectin serum levels 3 dpi were determined.

Results
Feed additives lowered *Salmonella* shedding (4.0 log CFU/g vs 5.1 log CFU/g; P=0.01) and ESBL/AmpC shedding (0.5 log CFU/g vs 2.0 log CFU/g; P=0.01) and increased Il-8 (275 pg/mL vs 228 pg/mL; P<0.05). A positive correlation was found between Il-6, Il-8, and Il1-β and between Il-1β and calprotectin (P<0.05). A negative correlation was found between Il-8 and diarrhea; Il-1β, Il-8, or IgA and Salmonella shedding; and Il-6 or Il-12 and ESBL/AmpC shedding (P<0.05). Il-1β tended to correlate to a higher feed intake, while Il-8 and calprotectin tended to correlate to a higher average daily gain (P<0.10).

Conclusion
In conclusion, feed additives decreased *Salmonella* and ESBL/AmpC shedding and increased Il-8 levels. High levels of immune and inflammatory markers correlated to less *Salmonella* shedding, ESBL/AmpC shedding, and diarrhea and tended to correlate to improved performance indicating that stimulating immune response during infection improves animal health and performance in weaned piglets.

Keywords: *Salmonella*, immune system, piglets, feed additives

VIII-5-025

The use of processing fluids compared to serum for determination the PRRS type 1 status of neonatal piglets on a commercial Dutch farm

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Introduction
For diagnosing early (vertical) PRRS-infections, a lot of piglets have to be bled. Bleeding new born piglets is stressful and time consuming which can only be done by well-trained people like vets. Recent findings from the US indicate the possibility of using processing fluids (PF) for diagnosing early PRRS-type 2 infections. Objective of this study is to compare PRRS-type 1 detection in serum and processing fluids of neonatal piglets during a field outbreak on a Dutch farm.

Materials and Methods
A 600 sow breeding farm with a recent PRRS-type 1 outbreak in the Netherlands was selected to compare the PRRS status of neonatal piglets by using PF and serum. Per week batch 30 piglets were bled by vena puncture at 2-4 days of life. In the same batches PF was collected. To collect PF, the testicles were put on a polyester 0.5 cm mesh grid. Samples were analyzed by PCR for the presence of PRRS-virus. Serum was tested pooled by 5 samples, PF was tested as one sample per week batch. When positive, the ORF5 sequence was analyzed.

Results
In total 4 out of 4 weekly batches, serum was positive for PRRS type 1 (Ct 31.0-36.2). In 3 out of 4 weekly batches, PRRS type 1 could be detected in PF (Ct 31.4-34.3). ORF5 sequence homology between the detected PRRS-type 1 strains and PRRS-Lelystad virus will be presented at the congress.

Conclusion
The use of PF for detecting PRRS in neonatal piglets is proven to be possible for PRRS-type 1 strains. However, not all PF samples were positive were serum was. The collection of PF by stockmen was easy and time efficient. In addition less PCR testing was used. With the use of PF, weekly farrowing batches can be monitored for PRRS status, saving time and money due to lesser amounts of PCR testing.

Keywords: PRRS type 1, processing fluids, PCR, sequence

VIII-5-047

A field-deployable RT-PCR system performs equivalently to real-time RT-PCR in detecting type 2 porcine reproductive and respiratory syndrome virus

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Introduction
Porcine reproductive and respiratory syndrome (PRRS) is characterized by reproductive failure of sows and respiratory problems of nursery and growing pigs. Rapid pathogen detection is important for disease management and control for PRRS. The POCKITTM PRRSV Reagent Set, a duplex RT-insulated isothermal PCR (RT-iiPCR) for type 2 PRRSV, was designed to target both ORF6 and ORF7 to increase test inclusivity. With excellent sensitivity (LoD95%, five genome equivalent) in a lyophilized format, the RT-iiPCR reagent set can work on the compact and field-deployable POCKIT Combo system (including a taco mini for automatic NA extraction and a POCKIT Nucleic Acid Analyzer for PCR; GeneReach) to provide simple and fast qualitative results on site.

Materials and Methods
In this study, in a PRRSV-stable pig farm free of other important diseases (e.g. PRV, FMD, CSF), 100 piglets from ten PRRSV antigen-free sows were tagged and divided into two groups; 50 were immunized with a PRRSV MLV vaccine at 1 week old, and the other 50 were not immunized. The presence of PRRSV in serum before and at 1, 2, 3, 5, 7, 9, and 11 weeks post immunization was monitored by testing 10 piglets by a previously validated laboratory. The samples were subjected to two PCR systems, i.e. total NA extraction by MagNA Pure LC2.0 (Roche Diagnostics)/real-time RT-PCR (rRT-PCR) on a LightCycler 480 (Roche Diagnostic) and by the POCKIT Combo system.

Results
Among the 160 samples tested, two of the 101 rRT-PCR-negative and four of the 57 rRT-PCR-positive samples were positive and negative by the duplex RT-iiPCR, respectively. Sequencing analysis of ORF5 of 11 samples of high PRRSV titers (determined by rRT-PCR) indicated the presence of wild type type-2 PRRSV strains. The rRT-PCR positive/RT-iiPCR negative samples all had a Ct > 37, suggesting the presence of relative low PRRSV titers. Kappa analysis indicated a 96.25% agreement (CI95%, 92.93-100%; κ=0.92), suggesting the two methods had equivalent performance.

Conclusion
The PRRSV-NA duplex RT-iiPCR and the field-deployable PCR system have potential to serve as a fast and sensitive tool for PRRSV detection at points of need, facilitating timely biosecurity management and disease control.

Keywords: type 2 PRRS, insulated isothermal PCR, on-site detection

VIII-5-050

New strategies for sampling piglets

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Introduction

During the last years several techniques have made diagnostics for PRRSV (porcine reproductive and respiratory syndrome virus) easier and cheaper (e.g. oral fluids, blood swabs or pooling samples). However, there are still opportunities for newer strategies to sample more animals with less effort. Taking advantage of processes or routines at farms such as the collection of tissues at piglet processing as a result of castration or tail docking or the collection of environmental samples is worth to explore.

The goal of this study was to evaluate the sensitivity of different diagnostic strategies to define the infectious status of a sow farm infected with PRRSV.

Materials and Methods

The study started 2 weeks after a PRRSV outbreak was reported in a sow farm and sampling occurred every three weeks for a total of 8 samplings over 24 weeks. At each time period, 10 litters were conveniently selected at processing (~ 3 days of age) before fostering. Processing fluids (PF) (fluids derived from tails and testes at castration) from the whole litter and individual serum samples from all piglets within the litter were collected. Wipes were collected from crate surfaces, udder skin from lactating sows and surfaces containing airborne particles deposited by gravitation.

Results

PF showed a sensitivity (Se) and Specificity (Sp) of 83% and 92% respectively when compared with the serum results used as gold standard. Surface and udder swab results showed a Se of 50 % and 42%, and Sp of 92% and 98%, respectively when compared to the individual serum results. PRRS RNA was detected in environmental and skin sow samples for up to 14 weeks after the outbreak.

Conclusion

PF are an effective sample to detect PRRSV in piglets, even after significant time since outbreak (~ 6 months) and the environment and the lactation sow may be a source for PRRSV infection in the farrowing environment.

Keywords: processing fluids, PRRS, diagnostics, surveillance

Ⅷ-5-055

Development of a Luminex multiplex assay for the detection of PRRS and PCV viruses and for PRRS vaccine differentiations in the US

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Introduction
Since its emergence in North America in the late 1980s, PRRSV becomes one of the most problematic pathogen in swine production systems, which causes significant economic losses in the US. The viral genome has undergone constant changes and new variants have evolved over time, which makes molecular diagnosis of PRRSV very challenging. There are four PRRS vaccines, Ingelvac MLV, Ingelvac ATP, Fostera and Prime Pac, have been used in the US. Differentiating vaccine strains from the field strains is important to guide and improve vaccine applications. The vaccine strains are very similar to some of the field strains and are primarily differentiated by ORF5 sequencing, which is expensive and time consuming. The Luminex xTAG assay is a bead-based nucleic acid detection that hypothetically can analyze more than 100 different targets in a single reaction.

Materials and Methods
In this study, a Luminex multiplex assay was developed to detect the vast majority of PRRSV-2 field strains, and to differentiate the four US vaccine strains. A collection of 694 full or near-full genome sequences of PRRSV-2 strains was analyzed. Two pairs of primers targeting the M and N genes were designed for general detection of viral strains.

Results
The coverage for each set is 85.4% and 91.2%, respectively, with a combined coverage of 98.1%.

Conclusion
Four pairs of primers targeting the nsp2 gene of vaccine strains were designed for vaccine strain differentiations. Testing on field samples and the four vaccine strains indicated that the assay detected all PRRSV-2 strains and identified each of the four vaccine strains accurately. To evaluate the limit of detections (LODs), a real-time RT-PCR was used for comparison. The LODs of the Luminex assay were equivalent to Ct 35.8 for MLV, Ct 33.2 for ATP, Ct 31.2 for Fostera, and Ct 36.1 for Prime Pac. The assay also included PCV2 and PCV3 detection with diagnostic coverage of 98.9% for PCV2 and 100% for PCV3 strains. LODs were equivalent Ct 36.4 by PCR for PCV2 and Ct 35.6 for PCV3. Work on adding SIV into the assay is in progress in order to achieve the goal of developing a comprehensive swine disease diagnostic assay.

Keywords: PRRSV, PCV, vaccine differentiation, luminex assay, real-time PCR

Ⅷ-6-004

B-eSecure: electronic system to measure and improve biosecurity on pig farms

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Introduction
Biosecurity procedures impact diseases such as PRRS, but applying and following biosecurity rules is often difficult. B-eSecure is an electronic system that besides external biosecurity, tracks and reports correct and wrong movements of people on farms and visualizes effects of biosecurity improvement on health status and production results. This PigChamp EU program is being piloted by MSD-AH in farms around the world and implementation in 2 Dutch farms is described.

Materials and Methods
Via installed tracking-devices, movements of people who wear personalized beacons are reported. The PRRSV status of sows, gilts, farrowing-, nursery- and finishing unit was determined and groups with circulating PRRSV defined as red vs groups without as grey. Movements from grey to red were defined as safe and from red to grey and between red as risk respectively unsafe unless a hygiene-lock was used between them (checked by locker devices). The % of correct and risk/unsafe movements per farm and person were reported monthly. Training and reports were implemented to reduce the amount of wrong movements. The effects on PRRSV prevalence and production parameter are monitored via regular diagnostics and management system.

Results
Farm (multiplying) 900 sows: PRRSV+ farrowing, nursery and rearing gilts. Risk/unsafe movements were reduced: September 27% vs October 23%. PRRSV prevalence in the farrowing units decreased by 50% and the amount of detected virus dropped 99% in nursery and farrowing.
Farm (breeding) 700 sows: PRRSV+ rearing gilts unit. Amount of wrong movements remained low at 7% in Oct. Extensive PRRSV monitor will be done every 4 months and 2 groups of mature-gilts tested monthly. No PRRSV was detected in October.

Conclusion
B-eSecure is very helpful for visualization, implementation and improvement of biosecurity procedures. Linking the program with PRRSV prevalence data and production results helps to reach and maintain a high level of biosecurity

Keywords: B-eSecure, PRRS, control

VIII-6-013

Questionnaire survey result of the current biosecurity situation in China pig farms by COMBAT

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Introduction
This study was the first survey report on the biosecurity situation of China's pig farm.

Materials and Methods
Survey tool was COMBAT which was an online- management biosecurity assessment software. All questions were developed as an online questionnaire and sent to customer's mobile phone by COMBAT App, and then were filled and submitted by themselves. The survey was started in July and ended in September 2017. Finally, 207 valid questionnaires came from 24 cities and provinces of China were collected.

Results
The results showed small scale pig farms (1-500 sows) were still in the majority with a ratio of 41.5%, farms with over 5000 sows only accounted for 10.1%. 75.3% pig farms were vaccinated with commercial modified live PRRSV vaccine (MLV) on sows, 57% pig farms used PRRS MLV on nursery or fattening pigs. The pig owners had a weak sense of biosecurity, 41.5% farms had no requirement to change clothing or boots. 58.0% farms required to clean and disinfect all compartments. 51.7% incoming gilts were PRRS positive stable and 15.0% gilts were shedding PRRS virus. Fortunately, 85.0% pig farms used semen from their own boars, but 13% semen was PRRS virus positive and 43% semen from PRRS antibody positive boars. 44.9% pig farms had no restrictions on vehicles, 31.9% vehicles were not washed and disinfected. Cross fostering was a daily affair, but 34.8% piglets were comingled with other age groups of different litters and moved to the same or different compartments. 34.3% pig farms cross fostered piglets at any time during suckling. 68.6% pig farms had weaned pigs in the farrowing house, and only 41.5% farms could achieved all in and all out. The density of pig farms was very high. 48.3% farms had more than one other farm in 1 Km radius. 66.2% pig farms even did not know PRRS status of the nearest neighboring pig farm.

Conclusion
This survey showed the most important risks parameters related to China's pig production.

Keywords: questionnaire, biosecurity, China, COMBAT

Ⅷ-6-020

Development of biosecurity assessment tool to evaluate biosecurity practices on Japanese commercial swine farms

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Introduction

Disease prevention through biosecurity measures is believed to be an important factor for improvement of the overall health status in commercial herd production. In US and Europe, a biosecurity assessment tool such as PADRAP and Biocheck have been developed to measure the biosecurity level. However, since production system and geographical condition vary between countries, it is required to develop the biosecurity assessment tool that fitted to the situation of each country. The objectives of the present study were to develop a biosecurity assessment tool, and to assess biosecurity level by using the tool on Japanese commercial farms.

Materials and Methods

A biosecurity assessment tool was developed by PRRS-Japan Elimination Team (P-JET) that consisted of clinical veterinarians and researchers. The tool was named as BioAsseT that has 131 questions and a full score of 100. BioAsseT consists of three sections (external, internal and monitoring biosecurities), and each section also has a full score of 100. In order to take account of importance of the different biosecurity aspects, each item of the 131 questions was weighted based on the scientific knowledge (high, intermedium and low). The results are presented lists of immediate priority items and well-done items as well as scores.

Results

By using BioAsseT, biosecurity levels were assessed on 103 farms. Mean total score (±SD) was 64.0±10.5, and the total score was normally distributed (Min: 39.2, 25th percentile: 56.3, Median: 64.2, 75th percentile: 71.5, and Max: 88.8). Mean score of external, internal and monitoring biosecurities were 58.9±13.8, 66.6±9.8 and 67.0±14.5, respectively. Total score increased as herd size increased, and score of three sections similarly increased as herd size increased ($P < 0.05$).

Conclusion

In conclusion, BioAsseT has been developed to measure and quantify biosecurity level on Japanese commercial farms. The present study found that there was a large variation of biosecurity level on commercial farms.

Keywords: BioAsseT, biosecurity, P-JET, PRRS

VIII-6-024

PRRS risk assessment of Dutch sow herds using COMBAT

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Introduction
COMBAT is a questionnaire tool to assess farm associated PRRSV risk factors by looking at variable risks: internal, external, management and location. In this overview we tried to have an insight in the most relevant risk factors for PRRSV infections of sow herds in the Netherlands, using COMBAT.

Materials and Methods
In 2017 at 31 farm locations in the Netherlands that housed sows a COMBAT questionnaire was performed. Each answer was classified ('Very high', 'High', 'Intermediate', 'Low' risk) and compared.

Results
Farm location was a very high risk in 68% of the cases; 87% have more than 4 pig farms within 5 km, 90% don't know the PRRS status of the nearest pig farm. External risks were (very) high in 56% of the cases; at 84% the pig transport vehicle was used at any farm, 61% without a need for drying the vehicle. Internal risks were (very) high in 46% of the cases; at 93% workers could walk freely between production areas. Management risks were (very) high in 62% of the cases; 90% puts lightweights to a younger age group and 87% had maximum 4 week quarantine period for incoming gilts.

Conclusion
This overview shows that PRRSV infection risks in Dutch farms are high. One may discuss if this is due to lack of knowledge and/ or motivation. High pig farm density is a considerable risk for PRRS infections. Nevertheless there was almost no knowledge on PRRS status of the nearest neighbor. The infection risk of mixing age groups is hardly recognized by farmers. For good PRRS risk factor advice every individual farm needs a custom made advice and a clear visualization of what might help. For that COMBAT can be used to visualize the farm PRRSV risk status and to discuss points of improvement.

Keywords: PRRS, risk factors, the Netherlands, questionnaire

Ⅷ-6-027

Meta analyses of biosecurity and management high risk scores in selected European countries based on COMBAT questionnaires

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Introduction
COMBAT (Comprehensive Online Management and Biosecurity Assessment Tool) is a new app developed by Boehringer Ingelheim Vetmedica to help farmers and veterinarians to evaluate and improve the level of biosecurity, pig flow and management procedures and benchmark against other farms.

Materials and Methods
This study is based on 258 COMBAT's (questionnaires) from England, France, Germany, Ireland, Italy, Netherlands and Spain. The relative risk is based on 55 questions divided in 4 categories: internal risks, external risks, location risk, management and pig flow. Feedback is given to each category and categorized as very high, high, medium and low risk, to facilitate discussion of behaviors and prioritize fields of importance.

Results
Examples of very high risky behaviors; -Location; majority of European farms does not know the status of their neighbors. -External biosecurity; 70% of farms pay little attention to flow and cleaning of vehicles between PRRS negative and positive farms. In almost 50% of farms, visitors and truck drivers can access the farm directly. -Internal biosecurity; in more than 48% of farms, gilts are in contact with PRRSV infected animals before entry. In 40-90% of farms, people are moving unrestricted around in the farm without boot or clothes change. 10-30% of farms regularly introduce PRRSV from outside via incoming animals. –Management and pigflow; PRRSV are moved between litters by sharing needles, sick pigs or handling boxes in 47% of farms. Mixing of pigs happens at several time points both before and after weaning. Still many farms introduce gilts directly into the sow herd.

Conclusion
PRRS status of neighboring farms mainly unknown, external biosecurity is often poor and a lot of farms are at extremely high risk to introduce PRRSV by animals and persons. Within the farms, PRRSV easily moves unrestricted around by animals, persons and equipment. COMBAT statistics allows addressing specific training needs, and highlight the need for whole herd protection in an environment with high risk of getting infected. Pigs and sows that are at risk of meeting PRRSV, should be sufficiently protected by a PRRS MLV vaccine.

Keywords: online, management, biosecurity, assessment tool, PRRS

VIII-6-031

Implementation of the 5 steps process platform for PRRS control in a farm in Spain

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Introduction
PRRS is one of the most damaging diseases in the swine industry, having negative effects typically affecting breeding herd reproductive parameters as well as pig productivity parameters. Controlling the infection is key to keep the systems producing at target levels and involves sow herd stabilization as well as an active pig protection.

Materials and Methods
The study has been conducted in a PRRS positive 750 sow farrow-to-feeder farm located in Spain. The 5 steps process considers defining goals, determining the status at the starting point, assessing system constraints, and developing, implementing and monitoring solutions. 200 gilts entered the farm before closing it and then two mass vaccinations of sows and gilts were done 3 weeks apart injecting intramuscularly 2 mL of Reprocyc PRRS EU®. The rest of the pigs older than 14 days were administered 1mL IM of PRRSFLEX EU®. Since then, every weekly piglet batch was vaccinated on a regular basis at weaning, the McRebel protocol was implemented and a sow and gilts quarterly vaccination was set up.

Results
Results of the means of the data before and after the implementation of 5 steps process are: Reduction in the abortion rate from 2.79 to 2.19. 0.879 more born alive piglets per litter. 0.595 more weaned piglets per litter. 1.76 more weaned piglets per sow per year. Reduction in the Nursery mortality from 4.82% to 3.65% Reduction in the fattening mortality from 6.16% to 4.07%.

Conclusion
The implementation of the 5 step process platform as well as the whole herd vaccination program implemented in this farm, had a significant positive impact on the reproductive and productive parameters. Regarding the financial impact the calculated return on investment was 12.1:1 for the intervention in sows and 6.0:1 for the intervention in pigs.

Keywords: vaccination, PRRSV, control

Ⅷ-6-041

PRRSV successfully handled with whole herd vaccination. Concept and biosafety analysis

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Introduction
Five farmers supplied by one piglet breeder increasingly complained of disease-susceptible and poorer-growing pigs and therefore an increased need for antibiotic treatment.

Materials and Methods
The piglet breeding facility is a 2 site production system with 480 sows, located in lower Saxony, Germany. The weaned pigs are kept in outdoor climate barns, 1.5 km away from the sows. The sows are vaccinated with an elaborate vaccination protocol including amongst others a regular vaccination against PRRSV (Porcillis® PRRS). The piglets are vaccinated against porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* (Ingelvac CircoFLEX® and MycoFLEX® simultaneously). In order to identify the underlying cause, 30 blood samples from sows and piglets were analysed for PRRSV (porcine respiratory and reproductive disease virus) and other agents. In a pool out of 5 blood samples of unvaccinated 30-kg piglets, PRRSV type 1 field virus was found. As from September 2015, a new vaccination scheme was established: the piglets received a new PRRSV vaccine (Ingelvac PRRSFLEX® EU) and the sow stock was treated with a new PRRSV sow vaccine (ReproCyc PRRS® EU) every 4 months. These measures were supported by a biosafety analysis with the application of critical external and internal biosafety aspects.

Results
From September 2015 onwards, the number of sows returning to estrus decreased from 17% to 10.6% when comparing the 16 month before the change in the vaccination protocol to those afterwards according to the sow planner software (db.Planer). The number of weaned piglets per litter was increased by 0.8 to 12.5. The suckling piglet mortality rate dropped from 18.2% to 12.5%.

Conclusion
The presented case report exemplifies how the combination of biosecurity improvements together with the implementation of a novel vaccination scheme including a whole-herd-vaccination and sow vaccination substantially reduced infection pressure by PRRSV, ameliorated animal health and enhanced performance parameters (as evaluated based on sow planner analysis).

Keywords: PRRS, vaccination, biosafety, software

VIII-6-044

Elimination of vaccine porcine reproductive and respiratory syndrome virus as part of PRRS elimination program using load-close-homogenize method

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Introduction
To eliminate vaccine virus from a breeding herd after a successful elimination of wild-type virus using Fostera® PRRS.

Material and Methods
An elimination project was implemented in a 2,800 sow farm which is part of a negative production system in Kansas, USA. This sow farm was laterally infected with wild-type PRRSV in January 2015. Outbreak management was successfully handled by vaccination of the breeding herd with Fostera® PRRS and herd closure for 210 days. The goal for this farm was to eliminate PRRSV as weaned pigs comingle with other negative sources. In February 2016, PCR positive results were identified in weaning-age piglets as part of routing monitoring; no clinical signs were observed in any of the production stages. ORF-5 sequencing showed 99% homology to Fostera® PRRS vaccine. After revisiting the plan of vaccination that was in place during the previous outbreak, we realized that in-place gilts development unit (GDU), which is a continuous-flow, was not homogenized (vaccinated) along with the breeding herd. Therefore, this was potentially the source for vaccine virus recirculation in the breeding herd. To address this situation, homogenization of breeding herd and GDU was implemented along with herd closure for 126 days. As part of the monitoring plan a set of metrics were defined as follow: Gilts-Flow-Interruption = Time between introduction of the last vaccinated and the first non-vaccinated gilts; Naïve-gilts-exposure = Time from last vaccination to exposure of non-vaccinated gilts to the rest of the breeding herd; Naïve-gilts-rollover = number of days gilts are flowing into the breeding herd. We followed AASV guidelines for PRRS breeding herd classification using PRRS PCR and ELISA tests in piglets at processing age and weaning age as well as replacement gilts.

Results
PRRS surveillance was implemented for 280-days post-introduction of naïve gilts without detection of PRRSV in gilts and piglets in farrowing house at processing and weaning-age confirming the success of the elimination program. Metrics generated during this project included: Gilts-flow interruption 77 days; Naïve gilts exposure 126 days and Naïve gilts rollover 280 days.

Conclusion
PRRSV elimination was possible using the right tools, metrics and surveillance methodology.

Keywords: PRRS elimination, Fostera PRRS

VIII-6-053

Economic value of porcine reproductive and respiratory syndrome type 1 stabilization on a large pig unit in Ireland

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Introduction
The clinical impact and cost of sub-optimal control of PRRSV type 1 is often underestimated. The aim of this study was to assess the changes to key production parameters on an endemically infected unit following the introduction of a PRRS control programme built around the '5 Step Process in which all pigs within the system were gradually vaccinated using recently launched PRRSV type 1 modified live vaccines. Biosecurity was also improved following the '10 Management Rules.

Materials and Methods
The study was conducted on a 1700 sow, two site farrow to finish system that had been infected with PRRSV type 1 but with minimal perceived clinical impact. Previously, the PRRSV vaccination programme consisted of an initial and a booster vaccine to gilts, followed by vaccination during subsequent gestations, using a commercially available PRRSV type 1 modified live vaccine (Porcilis® PRRS, MSD Animal Health). In mid-2016, a control and prospective elimination programme was instigated, consisting of 3-monthly mass vaccination of adults (ReproCyc® PRRS EU, Boehringer Ingelheim Ltd), weekly vaccination of piglets at weaning (Ingelvac PRRSFLEX® EU, Boehringer Ingelheim Ltd), a booster vaccine at 8 weeks of age and double vaccination of all gilts pre-service. By the end of 2016, all progeny in the system had been vaccinated and breeding and nursery herd stability was achieved. Key production parameters were compared for 2016 and 2017, for almost 90,000 pigs. An economic and statistical analysis of the data was performed.

Results
A significant improvement in average daily weight gain was achieved; 56 g/day from wean to slaughter in 2017, compared with 2016. Average carcase weight increased by 5 kg and average time to slaughter reduced by 2 days. The additional margin over feed was calculated as €5.15 per pig.

Conclusion
Improved control of PRRSV type 1 by a whole herd vaccination programme and improvements in biosecurity measures were cost effective, even where the impact of disease was considered to be low.

Keywords: PRRSV type 1, control, vaccination, value

VIII-6-055

Capture efficiency of filters against airborne pig pathogen models in an ASHRAE Standard 52.2 test duct

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Introduction
Airborne transmission of pig pathogens may be prevented in filtered buildings. Air filtration systems have to effectively capture pathogens or at least reduce the concentration to a non-infectious dose. As viruses, and bacteria often do not influence the aerodynamic diameter of airborne particles, it is difficult to design an air filtration system that is efficient, and economically suitable for pig producers. The objective of this study was to design and build a mixing chamber and to adapt an ASHRAE Standard 52.2 test duct for the aerosolization of microorganisms, and to evaluate the capture efficiency of different pre-filter and filter combinations for swine influenza virus (SIV), porcine reproductive and respiratory syndrome virus (PRRSV) and virulent *Streptococcus suis* serotype 2.

Materials and Methods
Five different air filtration systems were been studied, MERV 8 pre-filter + MERV 14 filter, MERV 8 pre-filter + MERV 16 filter, MERV 8 pre-filter + antimicrobial filter (5 layers), MERV 8 pre-filter + antimicrobial filter (10 layers), and MERV 8 pre-filter + antimicrobial filter (15 layers). Filter testing was conducted in an adapted ASHRAE 52.2 standard test duct. A Collison 24-jet Nebulizer generated airborne phages Phi6 and PhiX174 (influenza virus and PRRSV models), and *Streptococcus thermophilus* (*Streptococcus suis* serotype 2 model), airborne test dust (ISO 12103-1 A2 Fine Test Dust) was produced by a Fluidized Bed Aerosol Generator.

Results
The combination of MERV 8 pre-filter and MERV 16 filter had the highest capture efficiencies against swine influenza, PRRSV and virulent *Streptococcus suis* serotype 2 models.

Conclusion
A new platform has been designed and built to evaluate pre-filters and filters used in swine production to capture SIV, PRRSV and virulent *Streptococcus suis* serotype 2. Therefore, offering a novel evaluation method to prevent the spread of these pathogens between and within pig populations, reinforcing a component of farm's biosecurity measures.

Keywords: control and elimination

Ⅷ-6-056

Serology and molecular diagnostics for PRRS

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Introduction
Porcine reproductive and respiratory syndrome (PRRS) is characterized by reproductive failure of sows and respiratory problems of piglets and growing pigs. Since the first reports of its occurrence in the US and Europe at the end of the 1980s and early 1990s, PRRS has become endemic in most European countries and in North America and Asia. PRRS is considered one of the most economically significant diseases affecting the swine industry and great efforts are put into the control and elimination of the PRRS virus (PRRSV). Currently, two separate species of PRRSV are recognized: PRRSV-1 (previously genotype 1 or EU genotype) and PRRSV-2 (previously genotype 2 or US genotype). Both species are globally distributed and possess a high genetic diversity, with PRRSV-1 being further classified into four different subtypes. PRRS clinical presentation can differ greatly between herds and is mainly influenced by the virulence of the variant involved, co-infections and host susceptibility among others. Due to the variability of clinical signs in pigs infected with PRRSV and the absence of macroscopic and microscopic lesions of pathognomonic significance, appropriate diagnostic laboratory confirmation is required. Diagnostic techniques that focus on the direct identification of PRRSV and viral products include virus isolation, reverse-transcription polymerase chain reaction (RT-PCR), immunohistochemistry (IHC) and *in-situ* hybridization (ISH) methods. Indirect diagnostic techniques based on the detection of anti-PRRSV antibodies include ELISA, immunoperoxidase monolayer assay (IPMA) and immunofluorescence assay (IFA).

Results
ELISA and RT-PCR are the two techniques most commonly used in routine diagnostic investigations of clinical cases and are recommended methods for demonstrating freedom from disease at herd level and for surveillance purposes. Detection of anti-PRRSV antibodies in serum and oral fluid samples by ELISA and the use of RT-PCR for virus detection and identification are complementary diagnostic tools that provide insights into PRRS herd status, infection timelines and disease epidemiology, better informing swine practitioners and the diagnostic investigation.

Conclusion
This presentation discusses key aspects of PRRS diagnosis in commercial pig farms, with a focus on the use of ELISA and RT-PCR techniques, and reviews different factors that can influence diagnostics performance.

I -155

Dynamic distribution of classical swine fever virus in a subacute infection model

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Introduction
Classical swine fever virus causes significant mobility and mortality in pigs, resulting in a huge economic loss in porcine industry. Currently, subacute or chronic disease caused by moderately virulent CSFV strain infection is commonly observed in China.

Material and Methods
Herein, we infected pigs with moderately virulent CSFV strain (HeBHH1/95) to establish a subacute infection animal model. This animal model was then used to illustrate the correlation between viral dynastic distribution and tissue damage *in vivo*. Samples from tonsil, submandibular lymph nodes, spleen, kidney, lung, intestine, pancreas and ileocecal valve were collected at 1 day post infection (dpi), 3 dpi, 6 dpi, 10 dpi, 13 dpi, 20 dpi, 24 dpi and 28 dpi. Subsequently, viral RNA and protein dynastic distributions in these organs were analysed using CSFV ViewRNA *in situ* hybridization (ISH), immunohistochemistry (IHC), while histopathological lesions were identified using HE staining.

Results
The results showed viral RNAs were detected by ViewRNA ISH in tonsil, kidney, intestine, pancreas and ileocecal valve as early as 1dpi. Interestingly, viral RNAs were mainly observed in cells with secretion function, such as goblet cells, pancreas acinus, kidney tubules. At 3 dpi, viral RNAs were also detected in submandibular lymph nodes, lung bronchiole and spleen. Correspondingly, viral protein and histological lesion were observed in tonsil, kidney, intestine, pancreas at 1 dpi and in submandibular lymph nodes, lung and spleen at 3 dpi. Viral RNAs dramatically increased and were present in a wide range of organs at 13 dpi, following with more severe tissue damage in these organs.

Conclusion
Taken together, with the application of a novel ViewRNA ISH technology, we have discovered a direct correlation between viral RNA distribution and tissue damage in CSFV-infected pigs which provides key knowledge to unveil the pathogenicity of subacute CSFV infection.

Keywords: classical swine fever, ViewRNA ISH, dynamic distribution, subacute infection

Ⅰ-174

Eradication of classical swine fever in China: is it far away?

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Introduction
Classical swine fever (CSF) is a devastating disease of pigs. C-strain vaccine developed by Chinese scientists in the 1950s is a highly safe and efficacious lapinized attenuated vaccine against CSF, which plays an important role in the control and eradication of CSF all over the world. In the middle of the last century, China put forward the strategy to eradicate CSF. Now sixty years have passed, CSF is still endemic in most parts of China. What are the reasons? How far is the way to eradicate CSF in China?

Materials and Methods
This review summarized the recent situation concerning CSF in China and the world. The importance of the eradication of CSF in China and the favorable and unfavorable conditions for the eradication of CSF were discussed. This review also summarized the eradication experiences of CSF in Europe and America and discussed the ideas and plans for the eradication of CSF in China.

Conclusion
We believe that the eventual eradication of CSF in China is never so far away from us, considering its historical inevitability, strategic necessity, realistic possibility, and technical feasibility.

Keywords: classical swine fever, classical swine fever virus, lapinized attenuated vaccine C-strain, eradication, China

I -175

Research achievements of CSF diagnostic techniques

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Introduction
It is very important to develop CSFV antigen diagnosis methods with high specificity, sensitivity and easy-to-operate. CSF antibody detection is a key method to evaluate immune efficacy of CSF vaccines in China. Some important innovative breakthroughs have been achieved in OIE Reference Laboratory for CSF (OIERL for CSF) as following summaries:

Materials and Methods
CSF virus isolation, CSFV FAT/IPT, CSFV RT-nPCR, CSFV real-time RT-PCR, CSF Indirect ELISA antibody detection, CSF blocking ELISA antibody detection, CLIA Kit to Detect Antibody against CSFV.

Results
A monoclonal antibody of E2 protein was obtained, and it is used to optimize CSFV FAT/IPT method and improve the specificity and sensitivity of FAT/IPT. For detection of nucleic acid, several CSFV real-time RT-PCR methods have been developed. Among them, CSFV real-time RT-PCR could detect CSFV field strains, C-strain and Shimen strain, and it has become the first national standard for detection of CSFV RNA; CSF real-time RT-PCR MGB-P can detect field isolates specifically; C-strain real-time RT-PCR method can detect C-strain specifically, which could be used to control vaccine quality. CSF indirect ELISA antibody detection kit has been developed based on E2 protein which is expressed by the eukaryotic expression system. The sensitivity and specificity of I-ELISA Kit were 90.95% and 94.21% respectively. The coincidence rate of I-ELISA and NPLA was 92.62%. In addition, combined with E2 protein and monoclonal antibody, CLIA kit to detect antibody against CSFV is developed based on competitive ELISA principle. The kit is very stable and timesaving. The sensitivity is higher than I-ELISA, and the specificity is better than I-ELISA as well.

Conclusion
Until now, OIERL for CSF established, improved and performed CSF diagnostic tests with reference to the Manual of Diagnostic Tests and Vaccine for Terrestrial Animals, and also developed a series of novel diagnostic reagents independently. OIERL for CSF has taken part in the Inter-Laboratory Comparison Test for CSF organized by the EU/OIERLCSF 6 times from 2009-2016. All the results met the OIE/EU standards. A set of CSF diagnostic, monitoring and molecular epidemiological methods have been systematically developed. These diagnostic techniques provide technical support for CSF eradication in China.

Keywords: classical swine fever, diagnosis

I -138

Approaches for control of classical swine fever in Russia

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Introduction
Vaccination against classical swine fever (CSF) in Russia is mandatory. Vaccination practice has decreased the incidence of CSF, however the disease remains endemic. In order to contribute to potential eradication programs, new tools, including markered vaccine and diagnostics have been developed.

Materials and Methods
Molecular and reverse genetics methods were used to characterize Russian vaccine strain CS. The recombinant Erns and E2 surface glycoproteins of CSFV were expressed in baculovirus and purified using (His) 6 tags, monoclonal antibodies were obtained and epitope mapping performed. Blocking ELISA for antibody detection was developed based on the E2 and monoclonal antibodies. PCR was developed for CSFV detection and CSF/ASF differentiation. A collection of historic CSFV isolates were sequenced and analyzed.

Results
The CS live vaccine is based on the attenuated CSFV strain maintained in lamb testis cell cultures. The vaccine belongs to genetic subgroup 1.2. The genome is 12310 nucleotides long and has a 14 nucleotides insertion in the 3'-non-coding region. Sixteen unique restriction markers were found comparing to reference strains followed by development of PCR-restriction test to identify the vaccine from field strains. Majority of historic CSFV field isolates from 1981-1984 belonged to subgroup 1.2, isolates from 1995-2002 - to 1.1, and from 2004-2007 – to 2.3. A set of 28 hybridomas was obtained producing monoclonal antibodies to recombinant and native E2 protein. Five new non-overlapping linear B cell epitopes were mapped on the E2 using a set of 32 overlapping synthetic peptides. As a backbone for new live attenuated vaccines, we engineered an infectious clone of CS vaccine with mutations in the region specific for WH303 antibodies. This is a version of the CS strain that could be differentiated by serology. The purified E2 was tested as a subunit vaccine in immunization studies providing protection of pigs from lethal challenge. The Erns-specific antibodies were detected only after challenge with live virus, suggesting the use of E2-based vaccine for markered vaccination.

Conclusion
A complex approach has been applied to CSFV research with the purpose of development of eradication program including development of new vaccine candidate strains, subunit vaccine, immunochemical and molecular diagnostic tools.

Keywords: classical swine fever

I -024

First description of congenital tremor in a Swiss pig farm caused by atypical porcine pestivirus infection

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Introduction
In the last three years, several outbreaks of congenital tremor in piglets associated with atypical porcine pestivirus (APPV) infections have been described in Europe. Until recently, the associated mortality has been reported being low, and only limited information is available regarding pathogenesis, prevalence and epidemiology of APPV.

Materials and Methods
In a Swiss piglet-producing farm with 182 sows, an acute outbreak of congenital tremor was observed. Approximately six months before the outbreak, the farm started buying replacement gilts instead of breeding their own. Unexpectedly, in the first farrowing batch including purchased gilts, typical symptoms of congenital tremor occurred in litters only from homebred sows (n=6). The within litter prevalence in this batch varied from 25-83% and the mortality varied between 22%-71%. Two typical diseased piglets were submitted to the veterinary faculty for further examination.

Results
The clinical examination revealed that both animals suffered from congenital tremor. No significant lesions were observed during necropsy, but histopathological examination revealed multifocal vacuolation of mainly white matter that was most prominent in cerebellum, brainstem and midbrain and associated with suspected hypomyelination. An APPV RT-PCR targeting the NS3 and NS4B encoding regions of APPV was performed to confirm the diagnosis. For further evaluation, a continuous monitoring of the appearance of congenital tremor was established. A constant decrease of clinical signs and within-litter prevalence in the subsequent farrowing batches was recorded, finally reaching baseline values as observed before the introduction of the new gilts.

Conclusion
In this first case report of APPV in Switzerland, a very high mortality rate of piglets in affected litters was observed. It is most likely, that APPV was introduced and transmitted by purchased gilts.

Keywords: APPV, mortality, prevalence

Ⅰ-113

The first porcine Getah virus strain in China: isolation and identification

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Introduction
Getah virus (GETV), a member of *Alphavirus* has been associated with fetal death and abortion in pigs only in Japan and Korea but little is known in other countries. In this study, a non-specific segment was amplified by a pair of primers for amplifying CSFV S1 gene by a multiple RT-PCR from the abortive fetus clinically suspected as PRRS case in Henan, China. The sequence of the segment shared an identity of 99% with that of GETV. Thus the virus isolation and identification were conducted.

Materials and Methods
The MARC-145 monolayers cultured in flask were infected with the supernatant of liver and spleen from aborted fetus and incubated 1 h at 37℃ for absorption and then the supernatant was removed. By adding DMEM containing 2% FBS, the flask was incubated for an additional 48 to 72 h. After three blind passages, the isolate was purified by the plaque technique, detected by RT-PCR and observed by electron microscope. The complete genome sequencing of the isolate was also conducted.

Results
The results showed that a GETV isolate was successfully obtained, named strain HNJZ-S1, from aborted fetus of pigs in China. The isolate was consistent with the morphology and size of GETV. The virus gave rise to a titer of $10^{7.25}$ $TCID_{50}$/mL in Marc-145 cells 36 h post inoculation. The whole genome sequence of HNJZ-S1 (Acc. no. KY363862) is 11689 nucleotides in length and shared a similarity of 97.4% -99.3 % with that of 14 GETV reference strains reported in GenBank.

Conclusion
This study firstly confirmed that the GETV does exist in Chinese pig herd and the isolate is the only GETV strain from vertebrate to date in China. It has the potential importance of understanding the pathogenicity and transmission mechanisms of GETV in pigs and public health as well.

Keywords: porcine Getah virus, isolation, China

I -251

Identification and typing of astroviruses in wild boars

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Introduction

Astroviruses are usually associated with enteric diseases. These viruses were detected in humans and in many animals, including pigs. Several recent studies indicated that astroviruses were also found in feces of wild boars. We recently investigated rectal swabs of domestic pigs for astroviruses in our laboratory. Our project continues with study of the prevalence of porcine astrovirus (PAstV) in parenchymatic organs of wild boars in Slovakia. In addition, some of viral samples were typed to determine the astrovirus genotypes.

Materials and Methods

All together 200 samples of tissue homogenates (the cocktail prepared from spleen, kidney, and lymph nodes) were used in this study. A collection was represented by 99 old wild boars (>1 year) and 101 young boars (<1 year) sampled from different regions of Slovakia. The total RNA was isolated by Trizol method, cDNA was prepared using random hexamers, and the virus was detected by a semi-nested PCR using primers detecting wide spectrum of astroviruses. The amplicons were sequenced (n=13) in both direction by a commercial company. The phylogenetic tree was constructed by MEGA 6.0 program.

Results

The RT-PCR assay confirmed 28 PAstV positive samples, 13 in young and 15 in old wild boars, representing together 14% of positive infected animals. Phylogenetic tree indicated that 11 nucleotide sequences analyzed belonged to PAsV-2 (8 samples) and PAsV-4 (3 samples). Interestingly, additional 2 identical sequences collected from 2 wild boars in different geographic regions were close to chicken astrovirus.

Conclusion

This work confirmed that: Porcine astrovirus could be detected not only in feces of wild boars but also in other organs of infected animals. While domestic pigs were infected with all 5 astrovirus genotypes (our previous study), two astrovirus genotypes were confirmed in wild boars so far. Chicken astrovirus sequences were found in two wild boars but their origin in unknown.

Keywords: porcine astrovirus, genetic typing, wild boar

Ⅰ-011

Preparation and modification of African swine fever virus strain for CRISPR/Cas9 modification

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Introduction

African swine fever (ASF) presents a serious concern in world-wide production of pigs. In spite of numerous attempts and studies no vaccine is available against ASF. African swine fever virus (ASFV) developed different molecular mechanisms to evade host immune response. The aim of our project is the construction and investigation of biological properties of recombinant African swine fever virus (ASFV) strain lacking *A238L*, *EP402R* and *9GL* genes related to evasion or modulation of host immune response. The recombinant virus will be obtained using CRIPSR/Cas9 (**C**lustered ***R****egularly* ***I****nterspaced* ***S****hort* ***P****alindromic* ***R****epeats*) mutagenesis system.

Materials and Methods

The first step of this project was conducted using collected infectious material originating from 831 cases of ASF in wild boars and 104 outbreaks in domestic pigs to select 12 most representative ASFV isolates. The collected isolates were propagated using pig alveolar macrophages (PAMs) and continuous MARC-145 cells. All selected 12 isolates were tested using hemadsorption assay (HAD) and their titer reached from $10^{4.8}$ to $10^{6.0}$ hemadsorpitron units (HAU)/mL. Next the DNA of ASFV field isolates was extracted using High Pure Template Kit (Roche). The resulted DNAs were sequenced using next generation sequencing technology (NGS) on My Seq (Ilumina) machine. The obtained NGS sequences of ASFV field strains were assembled, and analyzed using Geneious R9 software (Biomatters, Auckland, New Zealand). The particular sequences were analyzed with application of appropriate databases including: Uniprot, Gatu (Genome Annotation Transfer Utility) and Viral Bioinformatics in Canada. Genomic modification using CRISPR/Cas9 system was applied using 2 designed sets of gRNA for each selected gene. The gRNAs along with Cas9 vectors were transfected into the infected MARC-145 cells and screened for modification using immunofluorescence assay and PCRs.

Conclusion

This world's first application of CRISPR/Cas9 system for edition of ASFV genome opens new horizons to explore the features of particular ASFV genomic regions as well as preparation of potential candidate for future vaccine against ASF.

Keywords: African swine fever, host immune response, evasion, CRISPR/Cas9, genomic modification

I -036

Influenza herd-level prevalence and seasonality in Midwestern US breeding herds

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Introduction
Influenza A virus (IAV) is a costly pathogen for pig producers and a risk to public health. Understanding IAV epidemiology is critical for control measures implementation. Information on prevalence variation over time and high risk periods identification is limited. Herein, we estimated IAV herd-level prevalence and seasonality in breeding herds, evaluated the correlation between IAV herd-level prevalence and meteorological conditions, and characterized IAV genetic diversity.

Materials and Methods
A cohort of 34 breeding herds located in the Midwestern US and with monthly IAV status in piglets prior to wean for over a 5-year period was selected. A farm was considered positive in a given month if at least one oral fluid sample tested IAV positive by RT-PCR. Seasonality was assessed combining autoregressive integrated moving average models with trigonometric functions (Fourier) as covariates. Meteorological conditions were gathered from local land-based weather stations, monthly aggregated and correlated with IAV herd-level prevalence.

Results
IAV herd-level prevalence had a median of 28% with a range from 7 to 57% and followed a cyclical pattern with prevalence increasing during fall, peaking in both early winter (December) and late spring (May), and decreasing in summer. IAV herd-level prevalence was correlated with mean outdoor air absolute humidity and temperature. Moreover, IAV genetic diversity was substantial over time with IAV isolates belonging to 10 distinct genetic clades from which H1 delta 1 and H1 gamma 1 were the most common. Twenty one percent of farms had 3 different clades co-circulating over time, 18% of farms had 2 clades, and 41% of farms had one clade. We showed that IAV is widespread, endemic and had a yearly cyclical pattern explained in part by air absolute humidity and temperature changes over time. Even though pigs are raised within environmentally controlled facilities, absolute humidity is not controlled, varies seasonally and has been associated with influenza survivability and transmissibility.

Conclusion
Our study highlights the importance of active surveillance to identify high risk periods that can guide the timing of IAV control measures in breeding herds. Our results also indicate the benefits of having a year-round surveillance to further understand IAV epidemiology, transmission and genetic diversity in pigs.

Keywords: herd prevalence, seasonality, absolute humidity, genetic diversity, breeding herds

Ⅰ-055

Swine influenza virus subtypes present in Mexico 2010 – 2017

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Introduction
Active swine influenza (SI) vigilance is important to identify the viral subtypes circulating in a country since pigs are susceptible to both avian and human influenza viruses, and the co-infection of two different viruses con result in the emergence of a new subtype by antigenic shift. The objective of the research was to identify the SI viruses subtypes present in Mexico.

Materials and Methods
Porcine samples (lungs, trachea and nasal swabs) obtained from the Western-Central Golf, North and Southwest regions of the country were processed, and the supernatant fluids inoculated in 9 days-old SPF chicken embryos incubated at 36℃ for 72 hours. Amnio-allantoic fluids were confronted with 2% chicken erythrocytes. Samples showing agglutination were collected and tested by RT-PCR to identify and determinate SI subtype.

Results
A total of 155 SI viruses were isolated. Lungs resulted as the most important source for isolation with 76.12%, followed by nasal swabs with 19.35% and by tracheal samples with 4.51%. The Western-Central Golf region resulted with the highest number of positive isolates with 58.06%, followed by the North region with 34.84% and finally the Southwest region with 7.09%. Finally, 47.74% of the isolates belong to the H1N1 subtype, 29.03% to the H1N2 subtype, 18.71% to H3N2 subtype and 4.52% to a mixture of two or more subtypes.

Conclusion
Among the regions analyzed, the predominant subtype in Mexico was H1N1, followed by H1N2 and H3N2 subtypes. The H1N2 subtype has been slowly displacing (since 2013) the H3N2 subtype. There is a presence of mixed isolates that might result in a new subtype as occurred in 2013 in the USA with the H3N1 subtype.

Keywords: swine influenza, subtypes, Mexico

I -081

Optimal influenza a virus vaccination: sows or piglets?

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Introduction
The objectives of this study were to evaluate the effect of sow mass vaccination with Respiporc FLU3, during a recent outbreak with H1avN2. Furthermore, a pilot study was performed to assess the impact of vaccination of new born piglets within the first seven weeks of life.

Materials and Methods
The two studies were performed in different herds. In Herd 1 an outbreak with a new subtype H1avN2 occurred. Sampling was performed on 4 batches of pigs before and after vaccination of all sows. In Herd 2 a whole stable of newborn piglets were subjected to either vaccination with Respiporc FLU 3 or injection with NaCl. Nasal swabs were collected weekly from 162 piglets from 11 different sows until seven weeks of age, and blood samples were collected at weaning. The bodyweight, clinical signs and nasal shedding of influenza a virus were compared between the two groups.

Results
The first study showed that the use of sow vaccination program significantly reduced the amount of viral shedding among the offspring and the number of individuals infected at one week of age. However, the total number of influenza shedding piglets was higher than before vaccination and the shedding period was significantly increased. Furthermore, no differences in incidence of respiratory symptoms (coughing, sneezing and nasal discharge) were seen between pigs from vaccinated and unvaccinated sows. The second study is still in progress, and the results will be presented at the conference.

Conclusion
In conclusion the results of the first study suggested that the use of sow vaccination program during an outbreak with IAV do not prevent infection and disease in weaned piglets and actually resulted in prolonged virus shedding. This emphasizes the importance of keeping the sow herd immunized prior to infection. The results of the second study will give indication if piglet vaccination provides a better alternative for the control of influenza in the farrowing and weaning unit.

Keywords: virology, influenza, vaccination

Ⅰ-084

Dynamics of porcine parainfluenza virus shedding in sows and their piglets

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Introduction
Porcine parainfluenza virus 1 (PPIV1) has become more prevalent in commercial swine production since its discovery in 2008. PPIV1 has been identified in the upper respiratory tract of pigs and clinical signs are typically observed at 10 to 21 days of age. The objective of this study was to characterize the dynamics of PPIV1 shedding in sows and their pigs from a clinically affected sow herd.

Materials and Methods
A 6,200 sow farm with clinical PPIV1 for 6 months was used. Sows and pigs were enrolled in the study based on parity of the sow. Oropharyngeal (OP) swabs were collected from sows at the time of farrowing and 33 pigs were enrolled at one week of age (WOA). OP swabs were collected from the pigs weekly from 1 to 7 WOA. A nasal swab was collected at 4 WOA for comparison with OP swabs. All swabs were tested for PPIV1 by PCR.

Results
None of the OP swabs from the 30 sows tested positive for PPIV1. One pig had a PCR positive OP swab at 2 WOA. By 3 WOA, all piglets tested positive. The pigs began testing negative at 5 WOA although 5 pigs were still positive at 7 WOA. At the 4 WOA sampling when both nasal and OP swabs were collected, nasal swabs were shown to have significantly lower CTs ($P<0.001$).

Conclusion
The PCR negative oropharyngeal swabs from sows suggested that shedding from the sows was absent or occurred at a low, non-detectable level. However, detection in sows may have been limited by the use of OP swabs. The pig data indicated that the ideal time for PPIV1 sampling of young pigs was at weaning and the first two weeks after weaning and that nasal swabs were more likely to yield higher virus levels compared to OP swabs.

Keywords: parainfluenza virus, shedding, PCR

I -153

Pathogenesis and transmission of a novel porcine parainfluenza virus isolate (MN25890NS/2016) in weaned and CDCD piglets

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Introduction
A novel *Paramyxovirus* detected in 2013 was designated porcine parainfluenza virus type-1 (PPIV-1). The objective of this study was to evaluate the pathogenesis of a PPIV-1 cell culture isolate in weaned conventional and cesarean-derived colostrum-deprived (CDCD) piglets.

Materials and Methods
Two animal studies using 30 conventional and 15 CDCD piglets, respectively, were conducted. Piglets were randomly allocated into challenge (Ch) and control (Neg) groups, with a contact (Cont) group introduced into the conventional Ch room on day 2 post-inoculation (DPI) or day 0 post-contact (DPC). Nasal swabs, oral fluids, and sera were collected. Piglets from the Neg and Ch groups were necropsied at DPI 0 and 5, and the end of the study (21 and 27 DPI in conventional and CDCD pigs, respectively). At necropsy fresh and fixed specimens including lung, trachea, nasal turbinate, bronchoalveolar lavage fluid (BALF) were collected for PPIV-1 nucleic acid detection by quantitative real-time RT-PCR and serum for antibody detection by serum neutralization assay.

Results
PPIV-1 nucleic acid was detected by rRT-PCR in nasal swabs from 29/30 pigs in the conventional and 10/10 from the CDCD Ch group at DPI 1 and 5/5 Cont conventional pigs at DPC 1. Viral shedding decreased at DPI 9 in both studies. The highest quantity of PPIV-1 nucleic acid were detected in the proximal and distal trachea. Macroscopic lung lesions consisted of minimal lobular tan consolidation in some conventional and CDCD Ch pigs. Microscopically, mild interstitial pneumonia was characterized by small peribronchiolar lymphocytic cuffs. Immunohistochemistry (IHC) detected abundant PPIV-1 signals in nasal turbinate, trachea, and bronchiolar epithelium. Neutralizing PPIV-1 antibodies were detected at 21 DPI in the Ch piglets.

Conclusion
Experimental PPIV-1 challenge in naive conventional and CDCD piglets caused a subclinical infection without morbidity or mortality. Macroscopic and microscopic lung lesions were minimal in spite of viral replication demonstrated by rRT-PCR and IHC in multiple tissues. In addition, PPIV-1 infection induced a humoral immune response based on neutralizing antibodies. These data suggest experimental PPIV-1 challenge causes a subclinical infection and viral replication that may support secondary infections of clinical relevance. PPIV-1 co-infection studies are needed.

Keywords: porcine, parainfluenza type 1, respiratory, pathogenesis

I -082

Deep-sequencing characterization of 16 rotavirus a outbreaks in sucking piglets in Spain

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Introduction

Group A rotaviruses are one of the main causes of diarrhea in pigs worldwide. In the last two years, in Spain there has been an apparent increase of reports involving rotaviruses in neonatal diarrhea outbreaks affecting even adult sows. In the present study, 16 epidemiologically unrelated outbreaks of diarrhea in suckling pigs and sows with Rotavirus A involved (as shown by RT-PCR) were investigated.

Materials and Methods

Nineteen positive stool samples were diluted 1:5 in sterile distilled water and total RNA was extracted by using the Trizol LS reagent. RNA was assessed for quality (A_{260} and A_{280}) and then submitted for library preparation and deep sequencing of RNA in an Illumina Miseq Platform. Samples yielding reads with a QC score >20 were accepted for further filtering. Reads were filtered against the 11 genes of Rotaviruses A using porcine rotavirus A sequences available at Genbank. Full-genome genotyping was done after blasting consensus sequences of each genome segment.

Results

The most frequent VP7 genotype was G9 followed by G3, G4 and G5. For VP4 the most frequent genotype was [P23] followed by [P7], [P13] and [P19]. The most common combination was therefore G9 [P23]. For all other genes the genotypes were common: I5-R1-C1-M1-A8-N1-T1/T7-E1-H1 (VP6, VP1, VP2, VP3, NSP1, NSP2, NSP3, NSP4, NSP5/6, respectively). However, the phylogenetic analyses indicated different significant clusters within all proteins but VP3 and NSP3. Interestingly, in one farm two different serotypes were found G9[P23] and G3[P7]. At present, the study of the past evolutionary events is being reconstructed.

Conclusion

Taken together, the results indicate a diversity of VP7 and VP4 genotypes circulating in pig farms. Given that the infection affected adults as well, it is tempting to hypothesize that these were new introductions.

Keywords: rotavirus A, diarrhea, genome sequencing

II-110

Diagnosis, prevention and control of porcine respiratory disease complex in an intensive pig farm

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Introduction
Porcine respiratory diseases complex (PRDC) is a general term for respiratory-related diseases caused by the interaction of many factors, including viruses, bacteria, mycoplasma, environmental stresses, etc. The viral pathogens mainly include PRRSV, SIV, PCV2, PRV and PRCV, and the mainly bacterial pathogens include *Streptococcus suis* (SS), *Actinobacillus pleuropneumoniae* (APP), *Haemophilus parasuis* (HPS), etc. PRDC is great threats to the pig industry. In July 2017, the pigs in an intensive pig farm were regularly experienced severe dyspnea, intermittent cough, grow slowly, low feed transaction rates, and severe acute deaths. In order to find out the etiology, the clinical symptoms of these pigs were observed and the clinical samples of 10 representative pigs were collected. A lot of cellulosic exudates and a wide range of visceral adhesions were found in the chest of those pigs.

Materials and Methods
Those samples were examined by PCR or RT-PCR, bacteria isolation and identification and drug susceptibility testing.

Results
The pathogens: PRRSV, PCV2, APP, HPS and SS, of which the serovars of APP was serovars 3, and multiple serovars of HPS (1 and 13) and SS (2 and 9) were identified in this case. And the drug susceptibility tests showed that APP, HPS and SS are all sensitive to enrofloxacin and florfenicol. So, this case was caused by the mixed infection of SS, HPS, under the lung damage of APP and the immunosuppression of PRRSV and PCV2.

Conclusion
According to the result, the sensitive drugs and targeted immunization procedures (such as stop using the vaccine of PRRSV) were used. After a period of days, the mortality of the farm significantly decreased, and the performance of pigs were greatly improved.

Keywords: porcine respiratory diseases complex, diagnosis, prevention

II -202

The European survey on lung lesions in slaughter pigs in 2017

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Introduction
Scoring of lung lesions in the slaughter pigs provides very important information about the respiratory health in the pig population. Lesions suggestive for previous *M. hyo* or *A. p*. infections and their scoring were described before. Scoring of those lesions allows quantifying the problems with enzootic pneumonia end pleuropneumonia. The aim of this survey was to collect the results of lung scoring performed in most of swine producing European countries in 2017.

Materials and Methods
Ceva Lung Program scoring methodology was implemented to score the lesions at the slaughterhouse. The results were collected from 19 European countries in the 12 months period from December 2016 till November 2017. The mean values and quartiles were calculated for % of lungs with *Bronchopneumonia* (%BP), % of affected lung parenchyma out of sick lungs (% parenchyma), % of dorso-caudal pleurisy (%DP) and APP index (APPI). For the two latter indicators the results from France were not included, because there they were not scored routinely.

Results
The total number of scored lungs was 325,624 from 2,918 reports with the average of 112 lungs per batch. The median value of %BP was 41.22% with the Q1=23.53% and Q3 62.91%. The median for % of parenchyma was 5.32% with the Q1=2.83% and Q3=8.41%. For % DP the median, Q1 and Q3 were 10.07%, 3.56% and 24.38% respectively and for APPI the corresponding values were 0.26, 0.09 and 0.61 respectively.

Conclusion
The data set from 19 European countries in 2017 shows very similar distribution of the values as the analysis made in 2016. This confirms CLP as a repeatable, relevant scoring methodology considering that fact that the amount of reports in 2017 increased by 50% compared to 2016. The incidence of especially EP-like lesions remains high despite the decrease for 8.5% *vs* 2016. The control of *M. hyo* infections seems still to be a major challenge.

Keywords: lung lesions, slaughter pigs

II-079

Absence of *Actinobacillus pleuropneumoniae* in semen from serologically positive tested AI-boars

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Introduction
Transmission of *Actinobacillus pleuropneumoniae* (Ap) between animals occurs mainly due to direct contact between pigs, due to nose-nose contact or uptake of nasal/oral fluids. To assess the risk of transmission of Ap by semen, first it is needed to know whether Ap can be detected in semen. The aim of this study was to evaluate the performance of a qPCR for the *ApxIVA* gene, on semen and test semen of seropositive but healthy boars for the presence of Ap.

Materials and Methods
To enable detection of the *ApxIVA* gene by qPCR in semen, the validated protocol for tonsil brush samples was adjusted, according to a validated protocol for detection of *Brucellosis* in semen. In short, DNA isolation was performed using the DNEasy kit. To assess the qPCR efficiency and minimal detection limit for Ap in fresh and undiluted semen a pooled semen sample was spiked with a 10-fold serial dilution of Ap in triplicate. Thereafter DNA isolation was performed and the qPCR was performed as described earlier (Tobias, 2012). Finally, 19 fresh and undiluted semen samples of serologically positive boars (by *ApxIV* ELISA and/or LC-LPS ELISA) were processed and tested by *ApxIVA* qPCR.

Results
The minimal detection limit of the *ApxIVA* qPCR in semen was >34 - ≤340 Ap DNA copies per reaction, when testing spiked semen. None of the semen samples of serologically positive tested boars (0/19) returned a positive qPCR result.

Conclusion
This study shows that *ApxIVA* qPCR testing of semen for Ap is feasible, but that detection limit increased from 5 copies / reaction in tonsil brush material to between 34 and 340 Ap DNA copies /reaction, which equals ~10^3 – 10^4 CFU /mL semen. As seminal transmission is already considered unlikely and in addition targeted screening of semen samples of serologically positive boars resulted in negative results, the risk of Ap seminal transmission by serological positive boars seems low.

Keywords: semen, artificial insemina, *Actinobacillus pleuropneumoniae*, *ApxIVA* qPCR, transmission

II-173

PleuroRes: Identification of genetic markers associated with enhanced resistance to porcine pleuropneumonia

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Introduction

Actinobacillus (A.) pleuropneumoniae is one of the most important respiratory tract pathogens in pork production distributed worldwide. It causes high economic losses due increased mortality and treatment in acute disease outbreaks or decreased performance in cases of chronic pleuropneumonia. Antimicrobial treatment and vaccination ensure only limited protection against the repercussions of the disease, as antibiotic may not reach sufficient levels on tonsils or inside sequesters and vaccination efficacy is hampered by limited cross protection between different serotypes. Therefore genetic disease resistance of the host holds much promise for sustainable disease control. Previous studies had discovered multiple QTL that could explain up to 22% of phenotypic variance. Based on these findings, this study aimed to identify genetic markers (QTN) for resistance to pleuropneumonia in a commercial German Landrace breed.

Materials and Methods

165 pigs from a segregating German Landrace breeding line were infected with *A. pleuropneumoniae* AP76 by a standardised aerosol infection protocol. Phenotypes were defined by a Respiratory Health Score based upon detailed clinical, sonographic and radiographic as well as on pathomorphological and microbiological examination results. 37 pigs of the most extreme phenotypes (susceptible / resistant) were selected and genotyped by sequencing (Next Generation Sequencing) in the context of a Genome Wide Association Study (GWAS).

Results

The GWAS identified three functional SNPs on three chromosomes, two of them in the range of the already identified QTL ($p = 10^{-12}$). Each variant explained 20-25% of the phenotypic variance of disease severity, in combination, up to 60%. The SNPs lead to functional modifications of genes that were proven to play a role in fundamental mechanisms of porcine pleuropneumonia pathogenesis.

Conclusion

Within this study gene markers for a genetic selection of pigs less susceptible for porcine pleuropneumonia have been developed and the genetic background for the host's susceptibility was confirmed. As favourable gene variants are segregating in commercial populations further work is needed to investigate prevalence of favourable and unfavourable gene variants in different breeds and populations and to verify the results of this study.

Keywords: *A. pleuropneumoniae*, genomic selection, breeding, QTN, next generation sequencing

II -097

Chimeric variant membrane surface lipoprotein (VlpA-G) as biomarker for early diagnosis of active *Mycoplasma hyorhinis* infection

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Introduction

Mycoplasma hyorhinis (MHR) is a common cause of polyserositis and arthritis and has emerged as an important contributor to mortality in nursery pigs. MHR has recently been identified as one of the main concerns of the U.S. swine industry. The antigenic diversity of MHR is generated by combinatorial expression and phase variation of multiple, size-variant membrane surface lipoproteins (Vlp) that are expressed in abundance. These Vlps undergo high frequency phase variation in expression and size providing a mutational strategy to generate diversity and evade the host immune response.

Materials and Methods

We investigated the suitability of a chimeric MHR Vlp recombinant protein as a biomarker for early detection of active MHR infection. Ten cesarean-derived colostrum-deprived pigs were inoculated with a MHR field isolate. Serum samples were collected on day post-inoculation (DPI) 0, 3, 7, 10, 14, 17, 21, 24, 28, 35, 42, 49, and 56. Pen-based oral fluids (5 pens, 2 pigs per pen) were collected daily throughout the study. The 7 known Vlps were synthesized and fused together to produce a chimera that was cloned and expressed in *Escherichia coli* BL21 (DE3). Recombinant VlpA-G protein was used to develop an indirect ELISA for serum and oral fluids.

Results

Clinical signs consistent with *M. hyorhinis*-associated disease, including lameness and loss of condition, were observed in 8/10 MHR inoculated pigs. Two pigs were euthanized on DPI 21 due to an inability to ambulate because of severe polyarthritis. MHR DNA was detected consistently between DPI 2-55 in oral fluids by qPCR. Lesions consistent with MHR, including epicarditis, pleuritis, arthritis, and peritonitis were observed at necropsy in 9/10 pigs. The earliest antibody response was detected between 10 (IgA) to 14 (IgG) DPI, with the number of antibody-positive pigs increasing by day post exposure. All animals were IgA positive by DPI 14 and IgG positive by DPI 28. The diagnostic and analytical specificity of the rVlpA-G ELISA was 100%, with no cross-reactivity detected against *Mycoplasma hyopneumoniae*, *Mycoplasma hyosynoviae,* and *Mycoplasma flocculare* pig antisera.

Conclusion

Based on these results, chimeric VlpA-G protein can be used as a specific and sensitive biomarker for early detection and diagnosis of MHR infections.

Keywords: *Mycoplasma hyorhinis*, variant membrane sur, Vlp, early diagnosis

II -054

Determining time to *Mycoplasma hyopneumoniae* stability in naive herds following whole herd exposure

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Introduction
Time-to-stability (TTS) has been defined for PRRS virus based on absence of clinical signs and sustained lack of viremia in weaning-age pigs (J Swine Health Prod. 2011; 19 (1):44-56). Wide use of TTS has not been applied to *M. hyopneumoniae* (Mhp) populations. Here we define TTS as the number of weeks to reach four consecutive monthly samplings. This study reports the evaluation of nasal swabs from weaning age piglets to determine TTS.

Materials and Methods
Farms A and B were independent Mhp negative 2500 sow breed-wean herds. Amidst acute respiratory signs, Mhp was confirmed by PCR in A on 44wk15 and B on 51wk15. Whole-herd exposure occurred for both resident sow populations and gilt replacements, followed by herd closure. Nasal swab PCR sampling began in weaning-age litters 13 weeks after Mhp confirmation for A and 8 weeks for B. Each sampling group consisted of 30 individual piglets, pooled in sets of three, no more than one pig per litter, with four weeks between sampling groups.

Results
Piglet sampling from A was 8/10 (80%) negative 13 weeks after Mhp was confirmed in the sow herd, but 100% negative 17 weeks after Mhp was confirmed in the sow herd. Sampling from B was 9/10 (90%) negative eight weeks after Mhp diagnosis, but 100% negative 13 weeks after Mhp diagnosis. Subsequently, both herds tested 100% negative for four consecutive monthly samplings, resulting in TTS of 30 and 26 weeks for A and B respectively.

Conclusion
Under these conditions, TTS was achieved in 30 weeks and 26 weeks for A and B respectively. TTS might be shortened if less time was invested between sampling groups. The sampling protocol is not sufficiently rigorous to suggest the downstream flow was 100% Mhp negative when stability was achieved; however, it is apparent Mhp immunity of the resident sow population was stable 26-30 weeks following aggressive whole-herd exposure.

Keywords: *Mycoplasma hyopneumo*, elimination, TTS

II-215

Longitudinal assessment of *M. hyopneumoniae* natural infection in gilts

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Introduction
Although the duration of shedding in experimentally infected pigs has been assessed, the epidemiology of *M. hyopneumoniae* (Mhp) in naturally infected gilt populations remains largely unknown. The objective was to describe the pattern of Mhp infection and persistence in a naturally exposed gilt population.

Materials and Methods
In this prospective cohort study, 63 unvaccinated gilts were sampled via laryngeal or tracheal swabbing weekly for 5 weeks and monthly afterwards until 340 days of age (doa). Serum samples were collected at 21 and 140 doa. During the farrowing stage, a tracheal sample was collected from study sows and 5 of their piglets. Nineteen gilts were discarded at different stages of the study due to not meeting the gilt selection criteria or death.

Conclusion
At weaning, 11% of the gilts tested PCR positive and 100% had Mhp antibodies. Between 28 doa and 140 doa detection was constant and the prevalence never exceeded 37%. Due to the decrease in detection at 170 doa (4,2%), side by side comparisons were completed between laryngeal and tracheal samples. Mhp was detected in 8.8% of laryngeal swabs from gilts compared to 42.2% of tracheal samples at 200 doa, and in 5.2% of laryngeal swabs compared to 52% of tracheal samples at 215 doa. This suggests that tracheal sampling could to be more sensitive compared to laryngeal swabs. Furthermore, although seroconversion was observed in 100% of gilts at 140 doa, 30% of those were PCR negative in laryngeal swabs after 8 sampling events. The prevalence of Mhp in tracheal samples decreased from 33% at 245 doa to 0% at 340 doa. The duration of bacterial shedding (detection) by gilts ranged between 30 to 284 days, with an average of 118 days. Four seropositive gilts (6.3%) remained PCR negative throughout the study. This limitation in the sensitivity of ante-mortem sampling should be taken into account when designing diagnostic protocols for Mhp. At farrowing, all sows and 100 of their piglets at weaning tested PCR negative. The assessment of the duration of shedding can aid the establishment of appropriate timing for gilt introductions and determining the duration of herd closures during elimination programs.

Keywords: tracheal sampling, enzootic pneumoniae, bacterial shedding, epidemiology, *M. hyopneumoinae*

II-152

The effect of gilt flow on *Mycoplasma hyopneumoniae* acclimation

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Introduction

Mycoplasma hyopneumoniae (Mhp) is the causative agent of enzootic pneumonia and a significant contributor to the porcine respiratory disease complex. Proper Mhp acclimation in recently introduced gilts is of crucial importance for sow herd stability in Mhp endemically infected farms. The objective of this study was to characterize the longitudinal Mhp colonization and seroconversion patterns in naive gilts recently introduced to positive farms.

Materials and Methods

Three sow farms located in the USA Midwest were enrolled in this study. Two of the three farms (A and B) practiced continuous flow, where gilts were co-housed at 65 and 45 days of age (doa) with older gilts exhibiting coughing immediately post-entry, and shared the same air space, regardless of age. The remaining farm (C) practiced an all-in/all-out flow, where gilts were housed in a separate air space from older gilts, and were co-housed with coughing culling sows at 130 doa. Two replicates, of 35 gilts each, were selected per farm and followed longitudinally (replicates were 60 days apart). Blood samples and laryngeal swabs were collected four/five times, approximately every 60 days. Samples were assayed for Mhp antibodies and genetic material using a species-specific ELISA, and real time-PCR, respectively. The last sampling event took place peri-farrowing in all 3 farms, thus 60 suckling piglets were sampled in farm B and C to evaluate potential sow-to-piglet transmission. Moreover, Mhp genetic variability was evaluated in gilts at all farms using MLVA typing.

Results

A similar real time-PCR Mhp detection pattern, as well as a similar seroconversion pattern were observed at all farms. No difference in the detection or seroconversion pattern was detected at the three farms regardless of the gilt flow type. Mhp genetic material was not detected in the last sampling event (prior to farrowing), including in gilts introduced to the farm at 130 doa. Also, no sow-to-piglet transmission was detected. However, 30.9% of the gilts were not detected positive by PCR during the study, which may have resulted from a possible acclimation failure, or failure to detect Mhp.

Conclusion

Additionally, the genetic variability analysis revealed a limited number of Mhp variants in gilts at the three farms.

Keywords: *Mycoplasma hyopneumoniae,* gilt acclimation, gilt flow

II-137

A study on the transmission of *Mycoplasma hyorhinis* in suckling piglets in two farm in northern Italy

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Introduction
The aim of the present study was to evaluate the way of diffusion of *M. hyorhinis* among suckling piglets in two farrow-to-finish farms in Northern Italy. *Mycoplasma hyorhinis* was longly regarded as a commensal, but recent studies suggest it can play an important role in PRDC, arthritis and polyserositis.

Materials and Methods
Two farrow-to-finish farm were included in the present study. Form each farm three sows each month for three months were selected. Nasal swabs were collected from each sow two days before farrowing. Swabs were tested for *M. hyorhinis* and *M. hyopneumoniae* using a real time Taqman probe PCR with internal control. After farrowing three piglets from each sow were selected and the same analysis were performed on sows and piglets on days 2, 8 and 18 after birth. An additional sampling was performed on piglets after weaning, at day 30.

Results
11/72 nasal swabs from 9/18 sows tested positive for *M. hyorhinis*. Only two sows tested positive more than once. Among piglets 1/54 swabs tested positive at day 2 (1.85%; 95% confidence interval (95% CI): 0.05-9.89%), 11/53 at day 8 (20.75%; 95%CI: 10.84-34.11%), 35/51 at day 18 (68.63%; 95%CI: 54.11-80.89%), 37/41 at day 30 (37/41=90.24%; 95%CI: 76.87-97.28%). Only three piglets tested negative after a positive sampling. The trend of positivity for *M. hyorhinis* suggest a discontinuous excretion by sows while piglets, once infected, tend to maintain *M. hyorhinis* positivity at the level of nasal cavity. Although the presence of *M. hyorhinis* from nasal cavities of sows appeared discontinuous, the diffusion of the pathogen among the litter seems to be quick, reaching at a prevalence around 90% by day 30 of life. The first piglets infected may play a role in the diffusion of the bacteria; the possibility of another route of shedding from sows (vaginal discharge, milk) is under evaluation.

Conclusion
According to our results, *M. hyorhinis* start to colonize nasal cavity of piglets during the first weeks of life and diffuses quickly among the litter.

Keywords: *Mycoplasma hyorhinis*, suckling piglets, sows

II-012

Efficacy of Vetmulin® for the control of *Mycoplasma suis* infections

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Introduction

Mycoplasma suis attaches and penetrates the erythrocytes and therefore leads to haemolytic anemia and reduced technical performances. A high health farrow-to-finish herd was suffering from pale suckling piglets despite an intramuscular iron injection. Simultaneously, impaired fertility and hypogalactia was noted in the sows. *Mycoplasma suis* was diagnosed by multiple positive quantitative PCR blood analyses and extreme low haemoglobin content values in preweaned piglets. As well in the farrowing units as in the nurseries, pigs showed lower growth and more secondary infections like diarrhoea and *Streptococcus suis* infections.

Materials and Methods

All sows and gilts were orally treated with tiamulin (Vetmulin®-Huvepharma®) at 10 mg/kg body weight/day for 14 consecutive days. The same dosage of Tiamulin was administered to the suckling piglets in milk cups for 5 days to stop the spread of the infection. Next to a close follow up of the haemoglobin values of the piglets, *Mycoplasma suis* PCR blood analyses were performed to evaluate the efficacy of the treatment. As purchased gilts showed positive PCR analyses at delivery, gilts were treated the first 14 days after arrival in quarantine. Technical data from the post-treatment period were compared to the pre-treatment period.

Results

A reduction of number of positives PCR samples and a complete recovery of normal haemoglobin values for the piglets was noted. Piglets in the farrowing units and nurseries showed a significant improvement of daily weight gain, lower mortality rate and a perfect general health status. The fertility of the sows restored.

Conclusion

Mycoplasma suis infections may have an enormous impact on rend ability of pig farms. The haemotrophic disease can perfectly be controlled by a strategic administration of Vetmulin®. Further follow up is required to check if the pathogen was eradicated or how long the infection can be controlled.

Keywords: tiamulin, *Mycoplasma suis*, Vetmulin

II-009

Current status of *Mycoplasma hyopneumoniae* infection in Chinese swine herds as determined by lung scoring at slaughter

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Introduction
Mycoplasma hyopneumoniae (M. hyo) is a major pathogen associated with porcine respiratory disease complex (PRDC) and enzootic pneumonia (EP). Slaughter check is a reliable way to assess the overall disease status of the herd. The purpose of this study was to investigate the prevalence of *M. hyo* and the severity of lesions in Chinese pig farms by performing lung lesion scoring at slaughter and establishing benchmarks between herds of different geographical origin, and also to investigate possible factors associated with *M. hyo* lung lesion severity at slaughter.

Materials and Methods
From 01/2016 to 07/2017, 10780 slaughter pigs were randomly selected from 104 farms in different regions of China. Lung lesions were visually scored through the MADEC method (Each lung lobe is scored on a scale from 0 (no lesion) to 4 (total inflammation of lobe)). Information about *M. hyo* vaccines or antibiotic usage, 1 or 2 shot vaccination were also obtained. The overall model and factors were analysed in anova and *T*-test analysis using Microsoft Excel (Data Analysis Toolkit).

Results
The percentage of *M. hyo* lesions and average Madec scores across the 3 regions are: North-46.15%, 3.70; East-46.00%, 5.67; South-59.90%, 3.56. For the different prevention methods, the percentage and average scores of *M. hyo* lesions are: Antibiotics-59.27%, 5.87; 1 shot vaccine-51.66%, 4.02; 2 shot vaccine-40.02%, 3.73. The average score between methods is significantly different (*P*=0.013). For 2 shot vaccine, the average *M. hyo* prevalence is 29.89% for M+Pac vs 41.54% for competitor vaccines (*P*=0.022) and lesion score is 2.56 vs 3.86 (*P*=0.049), respectively.

Conclusion
The results from this study indicate that *M. hyo* prevalence and lung lesion severity differs pending on the region. *M. hyo* vaccination significantly reduces lung lesion scores at slaughter and 2 shot vaccines performed better than 1 shot or antibiotics.

Keywords: PRDC, *Mycoplasma hyopneumoniae*, slaughter check

II-148

A survival analysis of *Mycoplasma hyopneumoniae* elimination protocols

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Introduction
Mycoplasma hyopneumoniae (Mhp*)* is an important respiratory pathogen causing economic losses in pig production. Elimination of this bacterium from swine herds results in increased productivity and animal welfare. Mhp elimination can be accomplished by different methods, which boast an overall high success rate. However, specific success rates may be different depending on the eradication method. Therefore, the objectives of this study were to determine the rate of herds remaining negative to Mhp after completion of one of two eradication protocols, and to compare the survival time between the two eradication protocols.

Materials and Methods
Fifty-six sow farms located in the Midwest in USA which underwent eradication programs constituted the cohort. Herd closure took place in 45 farms, and whole herd medication in 11 farms. All farms were followed up for a maximum of 155 months while they remained Mhp free. Mhp detection at farms was accomplished by using various diagnostic strategies. Two possible events were recorded: detection of Mhp (the event) or end of follow-up (censored observation). Time-to-event data were analyzed with Kaplan-Meier curves and Wilcoxon test, Cox proportional hazards model, and parametric methods. Moreover, the proportional hazards assumption was assessed using a simulation procedure. A sensitivity analysis to evaluate the assumption of independent censoring was performed as well.

Results
Time to detection of Mhp in 25% of farms was 8.2 months when whole herd medication was applied, and >155 months when herd closure was completed. The hazard of detecting Mhp in those farms with whole herd medication was 4.5 times the hazard in those farms with herd closure (95% CI: 1.1-18.1; $P<0.05$). The most parsimonious parametric model was exponential. The estimate for the survival time (negative to Mhp) in pig farms with herd closure was 4.45 times the survival time in those with whole herd medication (95% confidence interval: 1.11 – 17.78; P value<0.05).

Conclusion
Under the conditions of this investigation, Mhp elimination using herd closure significantly reduced the likelihood of detecting new cases of Mhp in swine farms, which makes it a highly suitable strategy to tackle this health problem in swine farms.

Keywords: *Mycoplasma hyopneumoniae*, elimination, survival analysis

II -203

Comparison of lung lesions in slaughter pigs vaccinated with different *M. hyopneumopniae* vaccines during a yearly survey in Germany and Austria

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Introduction
Piglet vaccination against *Mycoplasma hyopneumoniae* (*M. hyo*) is an effective method to tackle enzootic pneumonia (EP), reducing clinical signs and improving performance. The aim of this study was to compare lung lesions at slaughter from pigs vaccinated with four different vaccines against *M. hyo.*

Materials and Methods
From January 2016 to November 2017: 15,693 slaughter lungs were evaluated across Germany and Austria with Ceva Lung Scoring Methodology. Altogether 153 batches with a size superior to 30 lungs per batch were either assigned to the group vaccinated by Hyogen® (Ceva), One-Shot A, One-Shot B or Two-Shot C vaccines, with at least 20 batches per vaccination group. For each vaccine, mean lung values and incidences were recorded and compared statistically.

Results
The Hyogen® vaccination group had a EP-index of 1.013 (n=7,413), group One-Shot A 2.622 (n=3,730), group One-Shot B 2.569 (n=2,630) and group Two-Shot C 2.106 (n=1,920). EP-index was significantly lower (P<0.05) only for Hyogen® compared to vaccines A or B or C. Scar incidence was 8.19% for the Hyogen® group, 18.4% for group One-Shot A, 13.21% for group One-Shot B and 11.07% for group Two-Shot A (P<0.05 for Hyogen®*vs* A or B or C and for A *vs* B or C). Cranial pleurisy was at 15.65% in the Hyogen® group, 19.80% in group One-Shot A, 20.19% in group One-Shot-B and 19.1% in group Two-Shot A (P>0.05 for all combinations).

Conclusion
Under the conditions of this study the Hyogen® vaccination group showed the lowest EP index and also other EP-related indicators, suggesting that this vaccine induces superior lung protection in terms of *M. hyo* than the other three products. Similar results with this vaccine were previously reported from Spain. Furthermore, compared to the two-shot bacterin included in this survey, it requires less labour and is less stressful for the piglets.

Keywords: lung lesions, *Mycoplasma hyopneumoniae*, vaccines

II-100

Preparation of the ISCOMs of *Mycoplasma hyopneumoniae* P97R1 and its ability to enhance the effect of intramuscularly immunized live vaccine

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Introduction
P97 is the most important adhesion of *Mycoplasma hyopneumoniae*, which could effectively express only during natural infection. To investigate the ability of the recombinant protein of P97 R1 repeat region (P97R1) to enhance the effect of the *M. hyopneumoniae* live vaccine via intramuscular injection, immunostimulating complex containing P97R1 antigen (ISCOM-P97R1) was used to supplement the immune response to P97 and enhance the whole immunogenicity via its adjuvant activity.

Materials and Methods
ISCOM-P97R1 was prepared by dialysis method. The prepared ISCOM-P97R1, with a size of 30 nm to 40 nm, showed a cage-like structure under electron microscope. The entrapment rate of protein was 85.6%. The immunologic effect was evaluated in the mice model. One week after twice inoculations with the live vaccine adjuvanted with ISCOM-P97R1, high levels of serum antibody and significant lymphocyte proliferation against P97R1 and the whole *M. hyopneumoniae* proteins were detected. Protection against challenge were further tested on pigs.

Results
Pigs immunized with the live vaccine adjuvanted with ISCOM-P97R1 showed less lesions in lung, compared with the pigs immunized with live vaccine adjuvanted with ISCOM-matrix.

Conclusion
In conclusion, ISCOM-P97R1 showed significant enhancement on both the immunogenicity and protective efficacy of the live vaccine. It could be developed as a new adjuvant for the intramuscularly immunized live vaccine against *M. hyopneumoniae*.

Keywords: P97R1, live vaccine, ISCOMs, *M. hyopneumoniae*

II-115

Survey update on *Mycoplasma hyopneumoniae* acclimation of gilts in the Spanish swine industry

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Introduction
The introduction and management of replacement gilts is an important topic with regard to the control of *Mycoplasma hyopneumoniae* in the farm. Improper acclimation may result in colonization of the suckling piglets and increased *M. hyo* related respiratory disease in growing pigs. This survey is the second part of a previous one on the same subject in the Spanish swine farms.

Materials and Methods
The survey included 16 questions designed to identify which gilt acclimation methods for *M. hyo* are currently used in Spanish farms nowadays. The survey covered different farm related factors, demographics and detailed the structure of health protocols:

Results
The survey was completed by 116 production systems representing 639695 sows. The most important findings were: 42% receive naïve gilts into positive farms; 60% of farms have a replacement rate higher than 50%; 75% of farms practice late age acclimation, beyond 15 weeks of age; 79% of farms do not acclimate to the herd specific strain; 53% of farms use vaccines against *M. hyo* during the acclimation; 77% of farms do not perform diagnostics to verify an adequate acclimation; 63% of farms use antibiotics during the acclimation process.

Conclusion
While 88% of the producers or veterinarians are convinced that a proper gilt acclimation program plays a major role in the *M. hyo* stability of their farms, 54% of the respondents did not rely on their methods. Most of them do not have a clear definition of sow herd stability and the time needed for a proper acclimation. Besides, 77% do not verify the acclimation process of the gilts. Therefore, the survey reveals some opportunities to improve the acclimation process such as: the implementation of an early and efficient exposure method is needed; a “best practice” for *M. hyo* diagnostics has to be developed.

Keywords: *Mycoplasma hyopneumaniae*, gilt, acclimation

II-223

Hyogen® and Circovac® vaccines by separate or combined are effective against *M. hyopneumoniae* or porcine circovirus 2 experimental challenges

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Introduction
This study aimed to assess the efficacy of Hyogen® and Circovac® vaccines when administered separately or combined (ready-to-mix) by means of *Mycoplasma hyopneumoniae* (*M. hyo*) or PCV-2 experimental challenges.

Materials and Methods
Ninety six 2 week-old piglets seronegative against M. *hyo* and qRT-PCR negative but with low levels of antibodies against PCV-2 were allocated into 7 groups: non-vaccinated-non-challenged group (1, NV-NC), non-vaccinated-challenged groups (2 and 5, NV-C), separated vaccination groups-challenged (3 and 6, VS-C) and combined vaccination groups-challenged (4 and 7, VC-C). In the corresponding groups, Circovac® and Hyogen® were intramuscularly vaccinated (0.5 mL and 2 mL, respectively), separately or in a ready-to-mix combination; PBS was administered as control in rest of groups. Nine weeks post-vaccination (WPV), pigs from groups 2, 3 and 4 were intranasally inoculated with a PCV2b strain ($3x10^{5.4}$ $TCID_{50}$), and pigs from groups 5, 6 and 7 were endotracheally inoculated during 2 consecutive days with 5 mL of a M. *hyo* isolate ($10^{7.5}/10^{8}$ PCR50/mL). Animals were necropsied at 3 (PCV-2 groups) or 4 (M. *hyo* groups) weeks post-challenge. Parameters studied included qRT-PCR to detect PCV-2 in serum and PCV-2 and M. *hyo* ELISA antibodies at different time points and gross and histopathological lesions, PCV-2 antigen assessment by immunohistochemistry (IHC).

Results
The percentage of PCV-2 and M. *hyo* seropositive pigs from vaccinated groups was higher than NV groups from 3 WPV onwards in both challenge experiments. Both vaccinated groups reduced the percentage of PCV2 QPCR positive sera through the study when compared to NV-C group. PCV2 antigen was detected by IHQ in lymphoid tissues in 9 animals from NV-C group and in 2 from VC-C. NV-C had significantly higher number of tracheobronchial lymph nodes scored ≥1 than VS-C one and significantly higher number of animals with more than one lymphoid tissue with IHC score ≥1. Both vaccinated groups had lower number of animals showing macroscopic/microscopic lesions compatible with M. *hyo* than the NV-C group. No significant differences between vaccinated groups were found on macroscopic/microscopic lung lesions compatible with M. *hyo*.

Conclusion
Combined vaccination of Hyogen® and Circovac® produced similar effects on immune response, PCV2 viral load and post-challenge histopathological examinations as separated vaccinations.

I -052

The difference in pathogenic characteristics between PRV variant strains

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Introduction
A severe pseudorabies virus (PRV) variant that can cause high mortality in growing pigs in Bartha-K61-vaccinated pig farms has emerged in China since 2011. The pathogenic characteristics between five PRV variant strains named CH-18/2017, CH/SMX/2012, CH-17/2017, CH-7/2016 and CH/JXFZ/2014 were compared in BALB/c mice, aiming to screen a promising vaccine candidate of the variant.

Materials and Methods
Monolayers of PK-15 cells were infected with PRV at an MOI of 0.1 and they were collected at 36h after infection. 208 6-week-old SPF female BALB/c mice were randomly divided into 26 groups of 8 mice each. Groups 1–25 were each inoculated in hind footpads with 100μL containing 10^0, 10^1, 10^2, 10^3 or 10^4 $TCID_{50}$ of five variants, respectively. Group 26 were injected with DMEM as a negative control.

Results
Mice challenged at a dose of 10^3 and 10^4 $TCID_{50}$ all behaved typical pruritus at 3 to 5 days post infection (dpi). They scratched head with footpads and bit the injection site crazily. 3 to 6 dpi were peak period of death. There was no mouse died during 8 to 14 dpi. CH-7/2017 and CH-17/2017 behaved severest clinical symptoms. Mice infected with CH-17/2017 at a dose of 10^3 $TCID_{50}$ showed symptoms at 92.2 hours post infection (hpi) which was earlier than other strains. Mice infected with CH-7/2017 died at 170.94 dpi which is shorter than other strains. CH-18/2017, CH/SMX/2012 and CH/JXFZ/2014 were similar in these indices, which were significant different from CH-17/2017 and CH-7/2017. The LD_{50} of each PRV was calculated as $>10^{4.00}$ $TCID_{50}$ (Bartha-K61), $10^{1.92}$ $TCID_{50}$ (CH-17/2017), $10^{2.59}$ $TCID_{50}$ (CH-7/2017), $10^{3.20}$ $TCID_{50}$ (CH/SMX/2012), $10^{3.25}$ $TCID_{50}$ (CH/JXFZ/2014) and $10^{3.29}$ $TCID_{50}$ (CH-18/2017).

Conclusion
The variant PRV strains were different pathogenic to BALB/c mice. CH-7/2017 and CH-17/2017 were more virulent than other variants and can be considered to be candidates of the variant vaccine.

Keywords: Aujeszky's disease, Bartha-K61, variant strain, virulence

III-040

Porcine forebrain vacuolization: a novel condition of pigs associated with wasting

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Introduction
The term wasting does not imply a diagnosis by itself but is a clinical name to describe a physical condition characterized by growth retardation, usually of multifactorial origin. The present report describes an apparently new condition of pigs clinically characterized by wasting and vacuolization of the brain neuropil.

Materials and Methods
Since November 2016, an increasing number of farms in Spain have experienced wasting in nursery pigs. Animals are weaned in good body condition, and after 1-2 weeks, they start losing weight. Prevalence varies between 5-25%. Most affected animals do not die but are euthanized for humanitarian reasons. At necropsy, no significant lesions were observed, but serous atrophy and, occasionally, gastric ulceration. To investigate potential causes of this condition, 6 affected farms were studied (including 5 sick and 5 healthy pigs from each herd) by means of histopathology, transmission electron microscopy and detection of usual infectious agents.

Results
Pigs from 5/6 farms displayed significant difference in body condition and weight between affected and healthy pigs; healthy pigs from the sixth farm submitted had a very similar weight to that of sick animals. Besides the poor body condition, few gross lesions were observed in examined pigs, being erosion/ulcer of stomach and serous atrophy of the fat the most frequent ones. Histopathologically, the most consistent lesion was neuropil vacuolization of the prosencephalon, mainly located in the thalamic nuclei and in the transition between white and grey matter of the neocortex (24/30 in sick and 6/30 in healthy pigs). Interestingly, 4/6 healthy pigs that also showed this lesion were from the sixth farm. Electron microscopy of some of these sick animals showed preserved axons, with dilated myelin sheaths (interpreted as edema of myelin sheath). Porcine circovirus 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV) infections were ruled out.

Conclusion
Clinical and pathological investigations of these wasting cases in nursery pigs were associated with vacuolation due to edema of myelin sheath within the forebrain. Literature suggests this lesion type is linked to congenital or metabolic (toxic/deficiency) scenarios, but the precise cause of these cases is still unknown.

Keywords: wasting, brain vacuolization, emerging disease, dilated myelin sheaths

III-011

Network analysis on the contact structure between swine herds and feed suppliers during the 2014 porcine epidemic diarrhea outbreak in Canada

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Introduction
Porcine epidemic diarrhea virus (PEDV) emerged into Canada in January 2014 and it was suspected that a single feed company (FC) was the likely origin of the virus for the early cases in Ontario. The spray-dried porcine plasma (SDPP) from FC reproduced the infection under experimental conditions, however the complete feed did not. The aim of this study was to describe the contact structure of feed suppliers and porcine epidemic diarrhea (PED) case and control herds during early months of the 2014 Canadian outbreak.

Results
Separate case and control networks were generated to represent contact patterns with feed suppliers. The case network consisted of 21 network nodes (*n*=9 case herds; *n*=12 feed suppliers) and 161 connections. This network contained 2 weak components. The mean number of direct connections from feed suppliers to herds was 1.8 (range: 1-8). The maximum number of direct and indirect connections among feed suppliers and herds in a sequence was 9 (outgoing contact chain). The control herd network included 27 network nodes (*n*=13 control herds; *n*=14 feed suppliers) with 105 connections. This network consisted of 5 weak components. The mean number of direct connections from feed suppliers to control herds was 1.4 (range: 1-3). The maximal number of direct and indirect connections among feed suppliers and herds in a sequence was 4.

Conclusion
In conclusion, the case network had several network measures which suggested a higher degree of connectivity between feed companies and herds than the control network. FC played an important role in the network as it had the highest number of direct and indirect connections to case herds in the network. The control network was also more fragmented indicating that disease spread would be more difficult in this network due to fewer number of connected herds with feed suppliers compared to the case network.

Keywords: network analysis, feed, porcine epidemic diarrhea

III-045

The S gene is necessary but not sufficient for the virulence of epidemic PEDV strains

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Introduction

The recently emerged highly virulent variants of porcine epidemic diarrhea virus (PEDV) are the major cause of the global PED pandemic and have caused enormous economic losses to the worldwide swine industry. Remarkably, deletions, insertions or amino acid substitutions have been found in the spike protein (S) of the novel strains as compared to the classical strains such as CV777. The objective of this study is to determine whether the mutations within S gene are associated with the increased virulence.

Materials and Methods

By using reverse genetics, we generated two full-length chimeric infectious cDNA clones by swapping the S genes between the highly pathogenic strain BJ2011c and low pathogenic strain CHM2013. The viruses were rescued by transfection of recombinant BAC plasmids into Vero CCL81 cells and the virulence was tested in 2-day-old piglets.

Results

The animal studies showed that WT BJ2011C caused death of the piglets within 48 hours whereas the chimeric virus BJ2011C-$S_{CHM2013}$ carrying the S gene from strain CHM2013 showed very mild virulence and did not caused death of the piglets. On the other side, both CHM2013 and the chimeric virus CHM2013-$S_{BJ2011C}$ carrying BJ2011C S gene showed no virulence to piglets.

Conclusion

Thus, we conclude that the S gene is necessary but not sufficient to confer the enhanced virulence.

Keywords: PEDV, S gene, virulence

I -089

Porcine epidemic diarrhea virus-induced epidermal growth factor receptor activation impairs the antiviral activity of type I interferon

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Introduction
Porcine epidemic diarrhea virus (PEDV) causes acute and devastating enteric disease in suckling piglets and results in huge economic losses in pig industry worldwide. To establish productive infection, viruses must first circumvent the host innate immune response. In this study, we found that PEDV infection stimulated epidermal growth factor receptor (EGFR) activation, which has been linked to not only anticancer therapeutics but also antiviral signaling. Therefore, we determined whether EGFR activation affected PEDV infection by using activator or overexpression assay.

Results
The data showed that EGFR activation enhanced virus replication in both cases. We also found that specific inhibition of EGFR by either inhibitors or siRNA led to the decrease of virus yields. Further analysis revealed that inhibition of EGFR displayed an augmentation of type I interferon genes. We next observed that EGFR downstream cascade STAT3 was also activated upon PEDV infection. Similar to the case of EGFR, specific inhibition of STAT3 by either inhibitor or siRNA increased the antiviral activity of interferon and resulted in the decreased PEDV RNA levels, and vice versa. Data with STAT3 depletion in combination with EGFR activation suggest that the attenuation of antiviral activity by EGFR activation requires the activation of STAT3 signaling pathway.

Conclusion
Taken together, these data demonstrate that PEDV-induced EGFR activation serves as a negative regulator of type I interferon response and provides a novel therapeutic target for virus infection.

Keywords: PEDV, EGFR, STAT3, INF-I

Ⅰ-095

IL-22 suppresses the infection of porcine enteric coronaviruses by activating STAT3 signal pathway

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Introduction

Interleukin-22 (IL-22), a member of the IL-10 superfamily, is primarily secreted by T helper 17 cells, innate lymphoid cells (ILC), innate natural killer (INK) cells, and epithelial cells. IL-22 primarily targets nonhematopoietic epithelial cells and fibroblasts and plays essential roles in fighting against mucosal microbial infection and maintaining mucosal barrier integrity within the intestine. Given the critical role of IL-22 in epithelial regeneration, host defense, and pathology, IL-22 is an attractive target for clinical development in animals and humans. The most common pathogens of porcine viral diarrhea are porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV), and porcine rotavirus (PoRV). However, little knowledge exists on the ability of porcine IL-22 (pIL-22) to fight against viral infection in the gut.

Materials and Methods

In this study, we expressed and purified recombinant mature pIL-22 (mpIL-22). The antiviral activity against PEDV, TGEV, and PoRV was investigated by RT-qPCR, $TCID_{50}$ titration, western blotting, immunofluorescence.

Results

We demonstrated that mpIL-22 suppressed the infection of swine coronaviruses (PEDV and TGEV) and PoRV in the intestinal porcine epithelial cell line J2 (IPEC-J2) cells. mpIL-22 up-regulated the expression of the antimicrobial peptide beta-defensin (BD-2), cytokine IL-18 and IFN-λ. Furthermore, we found that mpIL-22 induced phosphorylation of STAT3 on Ser727 and Tyr705. Inhibition of STAT3 phosphorylation by S3I-201 abrogated the antiviral ability of mpIL-22 and the mpIL-22-induced expression of BD-2, IL-18, and IFN-λ. Moreover, mpIL-22 synergistically inhibit PEDV infection with IFN-λ.

Conclusion

mpIL-22 broadly inhibits swine enteric coronaviruses (PEDV and TGEV) and porcine rotavirus. Given that most coronaviruses infect epithelial cells in the respiratory and/or enteric tracts, it is worthwhile to further develop IL-22 as a potential therapeutic agent against the infection of coronaviruses.

Keywords: interleukin 22, coronavirus, STAT3

III-044

Early warning analysis of african swine fever propagation in Eastern Europe

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Introduction
African swine fever (ASF) is viral infection which causes acute disease in domestic pigs and wild boar. Although the virus does not cause disease in humans, the impact it has on the economy, especially through trade and farming, is substantial. Recent rapid propagation of the (ASF) from East to West of Europe encouraged us to prepare risk assessment for Poland and neighbouring countries. We perform early epidemic growth estimation and simulate landscape-based propagation. The values of chosen parameters have been estimated by regression functions, early detection growth estimation and spatial clustering.

Materials and Methods:
We analyzed 3230 observations (infection events) from February 2014 to November 2017 to their respective with time, longitude and latitude in Eastern Europe, where at least one house swine or wild boar case was reported. We take special attention to 25 Polish counties, which have been affected (until 31.01.2018). We provide several type of analysis: the early growth estimation, regression models, spatial clustering, propagation models. The early growth estimation can be easily done by matching incidence trajectory to the exponential function, resulting in the approximation of the force of infection. With these calculations the basic reproduction rate of the epidemic, the effective outbreaks detection and elimination times could be estimated. Early epidemic growth estimation indicates that to keep the epidemiological status quo will require a very fast response from veterinary services (less than one week after the detection to eliminate a single outbreak). We apply density-based spatial clustering to detect spatial clusters of infection events. We were able to distinguish Northern (Baltic States and Poland) and Southern (Ukraine and Balkan States) branches.

Results
In regression models, 380 Polish counties (poviats) have been analyzed, with 25 affected (located in Northeast Poland) for spatial propagation (risk assessment for future). We claim, that pig heads strongly explain both probabilities of introducing ASF as well as outbreak size (which is in contradiction to Estonian study). On the other hand, the forest coverage explains a little the arrival time of ASF (the same as in the Estonian study). Propagation model has been applied by taking into account: swine amount, disease vectors (wild boards), effective pork production chain, human failure to restrictions. This model will be equipped with decision support systems as a tool for veterinarians. We show which regions could be crucial for ASF propagation and which paths are the most likely.

Conclusion
ASF spreads from East to West of Europe with speed of around 200 km per year as a'wave'. Even if epidemiological situation in previously affected regions could stabilize in near future, the propagation will continue. However, we can predict most likely paths and attenuate the propagation.

Keywords: epidemiological modelling, computer-assisted risk assessment, ASF (African swine fever)

III-028

Identification and evolutionary analysis of atypical porcine pestivirus in China

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Introduction
Atypical porcine pestivirus (APPV), a novel found causative agent of congenital tremors (CT) in newborn piglets, has been widely identified in swine herds in the United States, Germany, Netherlands, Spain, Australia and most recently in south China. Here, we report the identification of APPV from piglets with acute CT and the prevalence and evolution of APPV in China.

Materials and Methods
Serum and tissue samples from piglets with acute CT as well as serum from the related sow were collected for APPV detection. Additional 285 swine serum samples collected from 93 farms in 22 Chinese provinces from 2015 to 2017, were also tested to screen the APPV prevalence in China. Viral RNA was extracted by using the E.Z.N.A™ Viral RNA Kit, followed with RT-PCR with SuperScript™ IV Reverse Transcriptase. The primers were designed based on E2 encoding regions from the available APPV genomic sequences in GenBank. The nested PCRs was carried out to detect the APPV. Further, the whole genome was amplified with 7 pairs of primers respectively. The sequences were aligned by using ClustalW in Lasergene software. And then the phylogenetic trees and molecular evolutionary analyses were conducted by MEGA 6 software using the neighbor-joining method with 1,000 bootstrap replications.

Results
An APPV strain CHheb1701 was successfully identified from the CT outbreak farm, and the sequence analysis indicated that CHheb1701 exhibited 83.1% to 93.0% nucleotide homology with previous APPV strains in GenBank. Phylogenetic analysis showed that CHheb1701 was clustered in the same branch with the American strain 000515, but it located far from branches with the Chinese strains GD1, GD2 and CH-GX. The E2 gene based RT-PCRs detection indicates a highly positive rate of APPV, including 25.3% (72/285) at samples level, 38.7% (36/93) at farm level and a highly prevalence of 81.8% (18/22) at regional level. The APPV genome could be detected as early as February 2015 in Henan Province. The molecular epidemiology analysis based on the E2 coding region showed highly diverse among strains.

Conclusion
An APPV strain CHheb1701 was identified. The molecular epidemiology analysis suggested that APPV has widely distributed in China with highly divergent characterization.

Keywords: APPV, congenital tremors, identification, evolution, China

III-018

Molecular detection, isolation and identification of Getah virus from pigs in 4 provinces in China

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Introduction
Getah virus (GETV), belonging to the genus *Alphavirus*, is a mosquito-borne enveloped RNA virus associated with some fetal diseases in horses and pigs in Japan, Korea and India. Recently, authors isolated the first Chines porcine GETV from a clinical illed pig herd in Henan province. This study was conducted to further understand the distribution of GETV in pig herds in China.

Materials and Methods
From 2016 to 2017, 801 samples of liver and spleen of clinically dead or illed pigs were collected from 231 pig farms in Shaanxi, Hebei, Henan and Anhui provinces, China, respectively. Samples were prepared for RT–PCR detection. Two genes, Cap and NSP3 of GETV were amplified by RT-PCR and some of samples positive to GETV were then used for virus isolation followed by a full length genome amplifying, sequencing and analyzing together with reference strains reported.

Results
RT-PCR detection showed that 37 samples (37/801, 4.62%) from 32 farms (32/231, 13.9%) were positive to GETV in 4 provinces detected above. A GETV isolate was obtained from the positive samples and named as HNNY-1 strain. All Cap, NSP3 and full length genome sequences and phylogenetic analysis indicated that the GETVs currently prevailing in Chinese swine herds were more closely related to the horse- and mosquito-borne strains isolated in Japan than mosquito- and pig-borne strains in China and Korea in recent years, and more genetically distinct from the primitive strain MM2021 in Malaysia and strains in Russia and Mongolia. The result also showed that all 54 sequences of GETV used in this study were divided into three gene groups, and 73.8% of them belong to group I, obviously with geographical and geochronological characteristics but no species-derived characteristic.

Conclusion
The study revealed that GETV existed widely in Chinese pig herds and it is necessary for people to pay attention to the pathogenicity and prevalence of porcine GETV and public health as well.

Keywords: porcine GETV, prevalence, China

III-057

Next-generation sequencing coupled with in situ hybridization: a novel diagnostic platform to identify emerging pathogens and new variants of endemic viruses

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Introduction
The objective of this study was to develop a diagnostic platform combining Next-Generation Sequencing (NGS) and *in situ* hybridization (ISH) technologies to specifically identify sequences of emerging pathogens in histological lesions.

Materials and Methods
Tissue samples from unsolved diagnostic cases at the University of Minnesota Veterinary Diagnostic Lab (UMN-VDL) were selected for next-generation sequencing. Extracted DNA and RNA were submitted to the University of Minnesota Genomic Center for library preparation. Based on the abundance of sequences detected by NGS for potential pathogens, specific ISH probes were designed. Formalin-fixed paraffin embedded tissues from cases submitted to the were retrospectively selected for ISH testing based on their PCR results for PCV2 (porcine circovirus 2), PCV3 (porcine circovirus 3), PPV2 (porcine parvovirus 2) and *Mycoplasma hyorhinis*. An ISH technique was developed to specifically target bacterial or virus mRNA.

Results
PCV2 and PCV3: Both viruses were predominantly observed in lymph nodes, spleen (white pulp), perivascular lymphohistiocytic infiltrates in the heart and peribronchiolar areas. There was no evidence of cross-reaction between the probes. The finding characterizes the ability of both viruses to replicate in the same tissue but with potential tropisms to different cell types during concurrent infections. ***PPV2:*** The distribution of PPV2 mRNA showed an association with the presence of lymphocytic perivasculitis and was located in the lymphocytic infiltrates and within the cytoplasm of endothelial cells. The present study is the first to describe the presence of PPV2 in association with systemic perivascular inflammation. This finding may represent an important advancement for understanding the potential role of PPV2 in systemic syndromes in nursery and finishing pigs. ***M. hyorhinis*-associated conjunctivitis:** Positive signals were observed associated with histological lesions on the conjunctival epithelium, and in the connective tissue where the lamina propria was exposed due to the ulcerative lesions.

Conclusion
The development of a NGS-ISH platform will incorporate the clinical-pathological significance into the sequencing technology through the *in situ* detection of the sequences identified by NGS. This platform will be available for swine veterinarians as a diagnostic tool and will help drive science-based decisions in the field, especially toward unsolved diagnostic cases.

Keywords: diagnostic, NGS-ISH, virus, swine, emerging diseases

III-004

Pigs and a crow residing close to pig farms share the same genotype of *brachyspira hyodysenteriae*

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Introduction
Corvid birds have been suggested to play a role in the transmission of *Brachyspira* (*B.*) *hyodysenteriae* causing swine dysentery in pigs. We therefore aimed to examine crows living in immediate vicinity to *B. hyodysenteriae* positive pigs for presence of *B. hyodysenteriae* and to determine if the isolates are genetically related.

Materials and Methods
Faecal swabs were obtained from pigs from two neighbouring *B. hyodysenteriae* positive free-ranging pig herds (10 swabs /herd) and from the intestinal content of four young crows (*Corvus corone*) hunted in the same area. The 24 samples were analysed by *Brachyspira* specific culture. Species identification was done using *nox*- polymerase chain reaction specific for *Brachyspira spp.* followed by restriction fragment length polymorphism. Sequence type (ST) of the *B. hyodysenteriae* isolates were determined using multi-locus sequence typing (MLST).

Results
B. hyodysenteriae was isolated from eight samples from one herd and from six samples from the other herd. One of the crows was also positive for *B. hyodysenteriae*. The eight isolates of the one herd and one isolate of the other pig herd belonged to ST196. The remaining five porcine isolates from the second herd as well as the corvid isolate were grouped into ST66.

Conclusion
This is the first description of *B. hyodysenteriae* in crows indicating that crows can potentially also contribute to the dissemination of *B. hyodysenteriae*. Further epidemiological studies are necessary to determine to which extend crows contribute to the maintenance and dissemination of *B. hyodysenteriae*. However, biosecurity measures should be taken to keep birds away from pigs herds.

Keywords: *Corvus corone*, swine dysentery, molecular epidemiology, transmission

III-014

Evaluation of pathogenicity of *Balantidium coli* in weaned pigs collected from several pig farms in southern, Vietnam

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Introduction
The study was conducted to determine the pathogenicity of *Balantidium coli* in weaned pigs collected from the field.

Materials and Methods
Total 61 pigs post weaning burdened diarrhea and diarrhea complicated with other clinical signs suspected infected with *B. coli* were collected. The parameters were evaluated included diarrhea status, body condition (1-4) of pigs and then subsequently faecal samples were collected for fresh fecal microscopic examination and total count (CPG) calculation by sedimentation method used Mc Master chamber and PCR confirmation. Then by routine necropsy, intestinal and other internal organs of the pigs were collected and evaluated the gross- and histopathological lesions to detect the invasion of *B. coli* into these organs.

Results
The prevalence of *B. coli* infection in diarrhea pigs was 73.77%. The prevalence of *B. coli* infection was significant higher in pig diarrhea complicated with arthritis/respiratory failure than with normal diarrhea (*P<0.01*). The mean intensity of infection of *B. coli* in the survey was absolutely high (CPG = 9,978 cysts and trophozoite). The intensity of *B. coli* infection in weaned pigs with poor body condition, gray feces diarrhea was significant higher than those of normal diarrhea and yellow feces diarrhea (*P<0.05*). The evidence of *B. coli* invasion in the large intestine was 54.09% and the small intestine (illeum) was 16.39%, an unsual site of infection. Intensity of infection at CPG ≥3,000 recorded gross- and histopathological changes prominently. Major gross lesions of white colon nodules and in particular the prominent invasion of *B. coli* into intestinal epithelium, gut associated lymphoid tissue, and submucosal lymphatic vessels were noted. The difference in the intensity of infection and the infectivity of *B. coli* on the intestine was significant (*P<0.01*).

Conclusion
This study determined the pathogenicity of B. coli *in weaned pigs in particularly cases of CPG>3000,* B. coli *can cause severe diarrhea in pigs through extensive invasion and ulceration into the mucosa,* intestinal epithelium, gut associated lymphoid tissue, and gut submucosal lymphatic vessels.

Keywords: *Balantidium coli*, pathogenicity, weaned pigs

I -131

The pathogenesis and risk for public health of zoonotic hepatitis E virus

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Introduction
To investigate pathogenesis of zoonotic hepatitis E virus and the potential risk of zoonotic HEV for public health.

Material and Methods
A literature search was conducted for relative publications to understand the HEV zoonosis, host range, transmission routes of zoonotic HEV to human, viral determinant of HEV host tropism as well as challenge faced by zoonotic HEV vaccine development.

Results
Since its confirmation as a zoonosis, food-borne transmission of swine HEV to humans has been a public health concern both for developing and developed countries. Meanwhile, the demonstration of a broad host range for zoonotic HEV suggests the existence of a variety of transmission routes that could lead to human infection. Moreover, anti-HEV antibody serosurveillance worldwide demonstrates a higher than expected HEV prevalence rate that conflicts with the rarity and sporadic nature of reported acute hepatitis E cases. It appears that both host factors and viral determinants were involved in determine the pathogenesis, host tropism and cross-species infection of different hosts. The newly identified quasi-enveloped HEV virion emerge as novel challenge for conventional subunit vaccine development against HEV.

Conclusion
Zoonotic HEV is an underestimated public health concern with unique characteristics requiring further investigation and development of improved vaccine against zoonotic HEV.

Keywords: hepatitis E virus, zoonotic HEV, viral pathogenisis, HEV vaccine

I -195

Patterns of HEV circulation on various types of pig farms in Poland

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Introduction
Hepatitis E virus (HEV) is one of the most common cause of acute hepatitis and jaundice in humans. Genotypes 3 and 4 have been isolated from both humans and pigs, and are recognized as zoonotic pathogens. The circulation of the virus in a pig herd varies depending on many factors. The objectives of the study were to demonstrate HEV circulation patterns on Polish pig farms.

Materials and Methods
Diagnostic materials were collected from 10 pig farms located in Poland. From each farm, 10 blood samples, 10 stool samples and oral fluid were collected from several age groups. The serum and faeces samples were pooled 10 to 1. Information on the size of the herd, type of production, hygiene level, health status and vaccination against PCV2 has been collected. For the detection of HEV RNA real-time RT-PCR was performed (Jothikumar et al., 2006).

Results
HEV RNA was detected in all of the 10 tested pig farms. In high and medium hygiene farms, the virus was mostly present in samples from pigs aged 15-17 weeks. In one of these farm HEV was found in serum of sows while materials from weaners and fatteners were negative. On the other hand, in small farms with a low level of hygiene and poor health status, the virus was detected as early as 5 weeks of animal life. In one of these farm HEV was present in samples from 5 to 17 weeks of life as well as in sows. The influence of the type of production system on the circulation of the virus was not observed

Conclusion
The results of this study indicate that HEV is common in swine in Poland but its circulation patterns differ between farms. Small family farms with low hygiene level are more vulnerable to long-term circulation of the virus between the age groups of the pig. It is still unclear which of these factors are primary and which are their consequence. Further research on the impact of risk factors and co-infection on the HEV circulation must be carried out.

Keywords: HEV, PCR, Poland

I -268

The diversity of the eleven gene segments of rotaviruses C among the host species

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Introduction
Rotaviruses (RV) are a major concern and cause of acute diarrhea in young piglets and children. The RV genome is composed of 11 segments of dsRNA encoding six structural viral proteins (VP1-VP4, VP6 and VP7) and five or six nonstructural proteins (NSP1-NSP5/6). Of the 10 RV (A-J) species, RVA, RVB, and RVC are found in diarrheic piglets. RVC is associated with acute diarrhea outbreak in nursing, weaning, and post-weaning pigs. The genetic diversity of RVA strains are well characterized while the genetic diversity of porcine RVC and other host species is poorly described. Therefore, the objective of this study was to characterize the genetic diversity of RVC among the porcine, bovine, and human host species.

Materials and Methods
The whole RVC genome was sequenced from 64 porcine samples from the USA. The newly generated porcine RVC sequences were aligned with the available RVC sequences in GenBank, and evaluated for genetic diversity. Descriptive statistics were summarized and genetic identities for each gene segments within the same species and among species were tested using non-parametric statistics with Kruskal-Wallis method. All statistical analyses were performed using R v3.4.3.

Results
The eleven RVC gene segments from pigs had statistically lower medians ranged (76.21 to 90.00% identities) than the other host species (*P*-value <0.0001). With the RVC gene segments for bovine and human, the median nucleotide identity ranged from 94.78-96.85% and 83.51-95.11%, respectively.

Conclusion
In conclusion, the median nucleotide identities for porcine RVC strains were lower than bovine and human RVC strains. With the high level of genetic diversity in porcine RVC strains, veterinarians need to match RVC strains in their preventive measure to mitigate and control acute diarrhea caused with RVC in nursing, weaning, and post-weaning piglets.

Keywords: rotavirus C, diversity, gene segments

Ⅰ-235

Different immunogenicity at post FMD vaccination: the role of porcine gut microbiome in immunogenicity

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Introduction
Since the foot-and-mouth disease (FMD), one of six OIE priority diseases, has swept away Korea, the government has turned to "FMD free with vaccination" as a livestock disease control strategy. Afterwards, the FMD vaccination has been performed nationwide with a penalty policy when the immunogenicity called the antibody positive rate against FMD was less than 80% among the herd. With the penalty policy, farmers claimed a lot because they experienced numerous discrepancies in field; pigs under the same management presented different immune responses. The study was performed to investigate why the pigs originated from the same sows displayed different immune responses under the same management practice and to develop the effective vaccination protocol through case analyses of 30 selected farms; good (17 farms) and bad (13 farms) pig farms.

Results
The result suggested that vaccination frequency, gilt vaccination, maternal antibody interference, vaccination before farrowing, clean needle use, right dose, and inflammation (non-specific host immune response) were found to be important roles in immunogenicity at post FMD vaccination. In particular, intestinal microbial analysis has revealed that gut microbiome play a significant role in the pigs' health status and furthermore in the proper immune formation. However, an agent for stress relief, vaccine storage and warm up before use, and syringe types (manual or automatic) did not significantly affect antibody formation rate.

Conclusion
It is expected that the porcine gut microbiome analysis and its use on management practice could improve general health promotion in herd eco-friendly.

Keywords: FMD, antibody positive rate, gut microbiome

I -263

Deletion in pseudoknots region of the 5′ -untranslated region implies a possible shift from cattle to pigs as the carrier for spread of serotype O foot-and-mouth disease virus

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Introduction
Foot-and-mouth disease virus (FMDV) is an extremely contagious viral disease of cloven-hoofed animals. In recent years, the serotype O FMDVs identified in China are mainly isolated from pigs, and a decreasing incidence of foot-and-mouth disease caused by serotype O FMDVs in cattle has been observed. Previous studies suggested that a 10 amino acids deletion in 3A protein was associated with an inability of CATHAY topotype FMDVs to cause disease in bovines in 1990s.

Material and Methods
In this study, we isolated an O/ME-SA/Pan Asia lineage FMDV strain O/GD/CHA/2015 that included an 86 nucleotides deletion in the pseudoknots (PKs) region of the 5'UTR of the viral genome. O/GD/CHA/2015 showed a pig-adapted characteristic that the virus could only cause clinical signs in pig and did not cause any clinical signs in cattle. To determine the role of the 86 nucleotides deletion in the pathogenicity of O/ME-SA/Pan Asia lineage FMDV in different hosts, a pair of FMDVs with complete PKs region or 86 nucleotides deletion in PKs region were constructed and rescued.

Results
We found that deletion of the 86 nucleotides in the PKs did not influence the pathogenicity of O/ME-SA/Pan Asia lineage FMDV in pigs, but contributed to the decreased infective ability of the virus to both bovine cells and cattle, indicating that the PKs region is an important determinant of host range. Meanwhile, we found that all the Cathay topotype FMDVs harbored a 43 nucleotides deletion in the PKs region. The role of the 43 nucleotides was also investigated by performing animal experiments using genetically engineered viruses. Deletion of 43 nucleotides also significantly decreased the pathogenicity of O/ME-SA/Pan Asia lineage FMDV in cattle.

Conclusion
Overall, our findings not only suggest that the PKs region deletion in serotype O FMDV genome occurred naturally in China and indicate the PKs region is highly associated with viral host range.

Keywords: foot-and-mouth disease virus, pseudoknots, Pan Asia, host range, viral pathogenicity

Ⅰ-159

Transmission of pig foot-and-mouth disease maternal antibody and it's negative effect on vaccine immunity

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Introduction
Maternal antibodies are transferred from sow to piglet, it's very effective in protecting neonates and infants pig against most infectious diseases. Vaccination of newborn piglets is problematic because of two unsolved problems: the presence of inhibitory maternal antibodies and the immature immune system of neonates pig. Foot-and-mouth disease (FMD) is a contagious disease of cloven-hoofed animals, it leads to enormous economic loss worldwide. One of the most important measures to control and prevent FMD is the vaccine immunity. How FMD maternal antibody transmit from swine to neonate piglets and the time point of first immune for neonate piglets are crucial in FMD prevention and control. Researchers have reported that pig maternal antibodies can inhibit seroconversion of veterinary vaccines such as *Erysipelothrix Rhusiopathiae*, pseudorabies virus, classical swine fever virus, influenza virus. The purpose of this study is to know how FMD maternal antibody transmit from swine to neonate piglets and the interference effect on FMD vaccine immune with the presence of FMD maternal antibody in piglets.

Materials and Methods
Sows were inoculated FMD vaccine one month before birth. FMD antibody in umbilical cord blood, colostrum and the milk were detected. Piglets which on 30, 43, 56 and 77 days old were grouped, and each group had 20 piglets. All the piglets were vaccinated with FMD vaccine and serum from anterior vena cava blood were collected and numbered. Second serum from all the piglets were gathered on 28 days after FMD immunization. FMD antibody were detected with liquid phase blocking (LBP) ELISA method.

Results
The only way of FMD maternal antibody transmit from swine to neonates is colostrum and the milk. Analysis of antibody data of FMD indicated that the higher maternal antibody level of FMD in piglets, the greater negative effect on FMD vaccine immunity.

Conclusion
So, there are two important measures for FMD prevention and control, firstly eating colostrum timely and secondly choosing first FMD immunization time point of piglets in the presence of maternal antibodies.

Keywords: FMD, maternal antibody, transmission, negative effect and vaccine

I -047

Seroprevalence of Senecavirus A in swine herds in the United States

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Introduction
Senecavirus A (SVA) is a picornavirus discovered in 2002 and documented retrospectively in United States' swine herds. SVA has been associated with idiopathic vesicular disease (IVD) and epidemic transient neonatal losses (ETNL). Outbreaks of SVA-IVD have been reported in several countries with increasing incidence since 2014. Serological studies shown that the proportion of seropositive animals in SVA positive farms did not differ between clinically and no clinically affected animals. However, there is not information available regarding the seroprevalence on USA farms without clinical IVD or ETNL associated with SVA.

Materials and Methods
Here we determine the seroprevalence of SVA in commercial swine in the USA. A total of 2,380 sows and 3,626 grower/finisher serum samples were collected from farms with no history of IVD, ETNL, and SVA diagnosis during 2016 at ISU-VDL. Thirty animals were selected from each unique site n=219 (n=88 sows; n=131 grower-finishers) representing 19 USA states. Sera were tested using a SVA-rVP1 ELISA and indirect immunofluorescence assay (IFA).

Results
The overall seroprevalence detected by rVP1-ELISA was 26.03% (CI ±1.76) and 10.97% (CI ±1.02) while by IFA was 25.72% (CI ±1.76) and 11.14% (CI ±1.02) for sows and grower-finisher, respectively. The between farm prevalence was 65.91% (CI ±9.9) and 21.37% (CI ±7.02) for sows and grower-fisher herds premises. The frequency of farms with a within herd prevalence ranging from 1-50% was 55% and 42.74%, ranging from 51-70% was 20.2% and 3.81%, and herds ranging from 71-100% was 16.85% and 9.9% for sows and grower-fisher herds premises. Clinically healthy sows and grower-finisher pigs possessed antibody against SVA with variable within herd prevalence.

Conclusion
The presence of antibodies in grower finisher indicates that colostral antibodies may persist for more than 6 weeks or animals were subclinically infected during the grower-finisher stage. These results strongly suggest that SVA is circulating sub clinically in USA swine population.

Keywords: Senecavirus, picornavirus, seroprevalence

II-143

Surveillance for *Streptococcus suis* needs to account for relatively low abundance of disease-associated strains relative to non-disease associated strains in carrier pigs

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Introduction
Streptococcus suis is a global zoonotic pathogen of pigs against which there is currently no comprehensive effective vaccine-based prophylaxis. Pigs may be colonised concurrently by strains that differ in their ability to cause disease. Disease prevention through widescale use of antibiotics at group-level is increasingly difficult to justify because of concerns over antibiotic resistance. Surveillance for disease-associated strains of *S. suis*, done on tonsil scrapes from healthy pigs prior to mixing with exclusion of carriers of disease associated strains, is one approach to setting up populations with reduced susceptibility to disease. Existing standard approaches to laboratory investigation of tonsil swabs for *S. suis* involve culture and then further characterisation of a limited number of colony picks (typically 3) to identify disease associated strains. We provide evidence that culture-based approaches to surveillance may underestimate the true positive rate.

Materials and Methods
Novel mutually exclusive genomic markers for disease-associated (DA) and non-disease associated (NDA) strains of *S. suis* were applied as quantitative PCR tests to tonsil scrapes from a total of 75 healthy 5 week old pigs drawn from 3 farms. The ratio of copy numbers of markers for disease-association versus non-disease association was calculated for each sample where both strain types were detected.

Results
A total of 51/75 samples were positive (above limit of quantitation (LOQ)) for the NDA marker while 14/75 were positive and >LOQ for the DA marker. 11/75 were positive for both markers with values that met quality controls. Calculated ratios of copy number per swab (DA:NDA) showed that DA strains were typically less prolific than NDA strains; 3 samples gave a ratio of 1DA:3NDA or lower, while 8 samples gave a ratio of 1DA:5NDA or greater.

Conclusion
This limited study indicates that DA strains co-exist with NDA strains in the tonsils of colonised pigs and that DA strains may frequently be overgrown by NDA strains. This observation is of importance in surveillance strategies employing culture with characterisation of a small number of colony picks since true carriers of DA strains may be overlooked. Typically, surveillance labs may pick only 1-3 colonies for characterisation.

Keywords: *Streptococcus suis*, surveillance, diagnostics

II-141

Genotypic characterization of *Streptococcus suis* isolated from diseased pigs in Brazil using PFGE

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Introduction
S. suis is one of most important pathogens in the swine industry worldwide, it is an emerging zoonotic pathogen that causes different systemic infections in swine and humans. The objective of this study was the genotypic characterization of *S. suis* isolated from diseased pigs in Brazil using pulsed-field gel electrophoresis (PFGE).

Materials and Methods
A total of 131 *S. suis* strains were studied. These were collected from swine with different systemic clinical signs, from nine different states, from 2001 to 2016. An identification by PCR and MALDI-TOF MS was performed. The serotyping was performed molecularly. The PFGE was performed with the restriction enzyme SmaI (New England BioLabs) and a DNA digestion at 25°C for 20 h was used. The PFGE fingerprint patterns were analyzed by a comprehensive pairwise comparison of restriction fragment sizes using the Dice coefficient, the isolates were considered in different pulsotypes when they differed by 4 or most bands and assigned into subtypes when they differed by 1 to 4 bands.

Results
The PFGE analysis resulted in 24 pulsotypes (P1-P24), with a tendency to cluster according to the year of the isolates; twelve pulsotypes were mostly comprised of strains isolated until 2003 and eleven pulsotypes were comprised of strains isolated between 2010 and 2016, the major pulsotype (P14) was formed by 52 of the strains studied, Among them, 67% (35/52) were comprised of strains isolated between 2001 and 2002, while 33% (17/52) were strains isolated in 2009, 2010 and 2016.

Conclusion
A relation between pulsotypes and isolation year was observed, indicating that genetic changes may have occurred over time within *S. suis,* but possibly a smaller percentage of strains maintained genotypic characteristics similar to previous years. These changes may be associated with phenotypic characteristics, such as antibiotic resistance and virulence factors, which will be further evaluated in the studied strains.

Keywords: *Streptococcus suis*, PFGE, pulsotype

II-159

A population-level study of antimicrobial resistance genes in *Streptococcus suis* in clinically heathy pigs in China and the UK

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Introduction

As an important zoonotic pathogen, the antimicrobial resistance of *Streptococcus suis* not only affects the global pig industry but also threatens the public health. 350 *S. suis* isolates obtained from the tonsil swabs collected from clinically healthy pig herds of 5 Chinese modern intensive pig farms, 5 Chinese traditional small pig farms and 5 intensive UK pig farms. Based on the whole genome sequences of these isolates, the prevalence and distribution of antimicrobial resistance genes (ARGs) were investigated.

Materials and Methods

A modified ARG-ANNOT database which includes 1817 ARGs was used to blast against the *S. suis* genomes for detecting ARGs. The minimum inhibitory concentration (MIC) of 15 antimicrobials of all *S. suis* isolates was determined by using the broth dilution method according to the recommendation by CLSI.

Results

In total, 32 and 14 kinds of ARGs were found in Chinese and UK database respectively. The overall carrying rate of ARGs of UK isolates were 90.55% while that of Chinese isolates was 100%. The Chinese isolates were from two different provinces located in the Central and South China, and compared with the UK isolates were from 2 counties, the geographical differences of the ARGs patterns were shown. The oxazolidinones and phenicols resistance gene *oprtA* was detected only in the Chinese collection, which may cause about 40% isolates resistant to florfenicol. There were 28 Chinese isolates were found resistant to penicillin, while there was no penicillin resistance isolate found in UK. Comparing the shifting of the ARGs during the feeding procedures, *S. suis* isolates contained more classes of ARGs in UK 20 weeks old isolates compared with 5 weeks, while that of Central and South China areas were the opposite. The same trend was also showed in the MIC test results.

Conclusion

The *S. suis* in clinically healthy pig herd carried considerable amount of ARGs. The emerging resistance of the *S. suis* to florfenicol may due to the associated ARGs and the situation of multi-drug resistant of *S. suis* is alarming.

Keywords: *Streptococcus suis*, antimicrobial resistance, clinically heathy pigs

II-014

The potential target proteins analysis of macrolide antibiotics inhibiting the biofilm formation by *Streptococcus suis*

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Introduction
Streptococcus suis (*S. suis*) is an important zoonotic pathogen that causes severe diseases in humans and pigs. Biofilms of *S. suis* may cause persistent infections and antibiotics resistance. In previous study, we have found that 1/4 minimal inhibitory concentration (1/4 MIC) of macrolide antibiotics (azithromycin, erythromycin, tylosin) can inhibit *S. suis* biofilm formation. Our major purpose in this study is to find the potential target proteins of macrolide antibiotics inhibiting the biofilm formation by *S. suis.*

Materials and Methods
By using the iTRAQ strategy, we compared the protein expression profiles of *S. suis* grown with 1/4 MIC macrolide antibiotics treatment and with no treatment. According to the ratio >1.2 or <0.8 (P <0.05) protein screening, 79 (erythromycin), 174 (azithromycin), 171 (tylosin) differentially expressed proteins were identified.

Results
The results indicated that : Cell surface proteins played an important role in biofilm formation; Macrolide might a□ect adhesion of *S. suis* by increasing capsular polysaccharide (CPS) content, then affect the biofilm formation; The 50S and 30S ribosomal were differentially expressed, it may be the macrolide inhibiting the protein synthesis of ribosome, thereby inhibiting the biofilm formation; ABC superfamily ATP binding cassette transporter was differentially expressed, it coded by *ComAB* gene which participated in the quorum sensing (QS) system, the macrolide may affect the QS system by interfering this protein, ultimately affecting biofilm formation.

Conclusion
In summary, the macrolide may inhibit biofilm formation by affecting cell surface proteins, CPS synthesis proteins, 50S and 30S ribosomal proteins and QS system-related proteins. In other words, macrolide affects *S. suis* biofilm as a result of multiple protein targets and integrative factors.

Keywords: *Streptococcus suis*, biofilm, macrolide, iTRAQ, protein target

II-189

Respiratory pathogen spectrum in affected and non-affected weaners from piglet producing farms with recurring respiratory disease

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Introduction

Respiratory diseases play a major role in raising weaners. Often they occur as multifactorial disease complex with combined underlying sub-clinical and secondary infections. Thus, for a sustainable treatment it is important to identify the main pathogens involved. In this study, bronchoalveolar lavage (BAL) samples were taken on piglet producing farms with recurring respiratory disease problems from apparently healthy and affected weaners to compare their spectrum of respiratory tract pathogens.

Materials and Methods

Piglet producing farms (n=30) with preceding recurring respiratory diseases in weaner units were visited twice. The first visit (A) took place when the health status of the weaners was not affected, the second visit (B) when a respiratory disease outbreak required antibiotic treatment. On both days BAL samples were taken from four (A) or three (B) weaners, respectively. These samples were examined by bacteriological culture on blood and chocolate agar and by PCR for the presence of respiratory tract pathogens.

Results

On visit A 72.5% of the BAL samples and on visit B 93.0% of the samples were positive for specific respiratory tract pathogens. On both days *Haemophilus (H.) parasuis, Bordetella (B.) bronchiseptica, Pasteurella multocida* and *Streptococcus suis* were the main isolates detected. Although the spectrum was not significantly different between both examination days, the quantity of bacterial load differed between visit A and visit *B. H. parasuis* was detected in 50.8% (A) and 76.4% (B) of the samples; *B. bronchiseptica* in 14.2% (A) and 23.6% (B) and *Mycoplasma hyopneumoniae* in 10.8% (A) and 15.3% (B). The quantity of bacterial growth within the positive samples was significantly higher in samples from visit B. Additional infections not detected on visit A occurred in 18.3% on visit B, here mainly influenza-A-virus and PRRSV were identified.

Conclusion

This analysis shows that in cases of preceding problems with respiratory diseases often the same pathogens are present in periods with and without disease symptoms only varying in number. Therefore it is necessary to identify these underlying bacterial respiratory tract pathogens as major targets for antibiotic disease treatment to achieve a sustainable control of recurring respiratory diseases.

Keywords: respiratory disease, bronchoalveolar lavage, PRDC, pathogen diversity

II-199

Molecular characterization of *Haemophilus parasuis* field isolates by serotyping PCR (sPCR) and virulence-associated autotransporters PCR (*vtaA*-PCR)

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Introduction
Haemophilus parasuis (HPS) isolates have been classified into 15 serotypes by gel immunodiffusion and hemagglutination. The accuracy of these methods is limited. The objective was to characterize HPS field strains by a PCR-based serotyping method, and investigate potential associations between serotypes and virulence of HPS strains.

Materials and Methods
One hundred isolates from diseased pigs in 14 US states, over a 4-year period were tested. The age of the affected pigs varied from pre-weaning to adults, but most were less than 10 weeks of age. Fifty-five isolates were recovered from serosa surfaces, 30 isolates were obtained from lung of pigs with polyserositis and 15 isolates were recovered from lungs of pigs with pneumonia.

Results
Serotypes 4 (25%) and 1 (21%) were the most commonly detected serotypes, followed by serotypes 13 (16%), 5/12 (14%), 7 (10%), 2 (9%), 14 (3%) and 6 (2%). Previous studies have reported serotypes 4, 13, 5 and 7 as the most frequently detected serotypes in pigs with HPS-associated disease in North America. Serotypes 1 (24%), 13 (22%) and 5/12 (16%), were most frequently detected from systemic sites, whereas serotype 4 (37%) was most frequently observed in isolates originating from lung samples. The sPCR does not differentiate between serotypes 5 and 12. Currently optimization of a new PCR is underway to differentiate those isolates and results are pending. Serotypes 3, 8, 9, 10, 11 and 15 were not found. Previous studies have also found a low carriage of these serotypes in pig populations.

Conclusion
To date, 70/100 isolates so far tested were positive for the HPS virulent *vtaA* gene, suggesting a potential high level of carriage of virulent strains by pigs. Interestingly, serotypes 6 and 7, previously considered avirulent, originated from systemic sites of pigs with polyserositis, and 3/10 serotype 7 isolates were *vtaA* positive strains, suggesting that within this serotype not all strains are avirulent. No correlation was found between serotype and age, state or site of isolation. More studies are needed to elucidate associations between particular serotypes and virulence. Serotyping can aid in the development of effective vaccine programs and minimize disease by improving pig flow management.

Keywords: *Haemophilus parasuis*, serotyping PCR, *vtaA*-PCR, molecular characterization

II-045

Genetic characteristics of *Pasteurella multocida* isolated from pigs in China

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Introduction

Pasteurella multocida is a leading cause of porcine respiratory disorders worldwide. This Gram-negative pathogenic bacterium has been assigned in to five capsular genotypes (A, B, D, E, F) and/or eight lipopolysaccharide (LPS) genotypes (L1-L8), respectively. While it has been reported that both capsular types A and D are the main epidemic *P. multocida* capsular genotypes in pigs, the predominate LPS genotypes are still unknown. It is also lack of the knowledge about the multilocus sequence typing (MLST) genotypes as well as the virulence factors (VFs) associated genes of the epidemic *P. multocida* in pigs. In addition, the phylogenetic relationship of *P. multocida* recovered from different hosts/diseases remains an unsolved problem. In this study, we figured out the capsular genotypes, LPS genotypes, MLST genotypes, as well as the main VFs-associated genes of *P. multocida* isolates from pigs during the past four years (2014-2017) in China.

Results

Our results showed that though genotypes A and D were still the predominate *P. multocida* capsular types in pigs, other types of capsule such as genotype F and the “nontypable strains” were emerging. The genotypes L3 and L6 were the epidemic LPS genotypes, and L6 was more predominate than L3. While a number of MLST genotypes were identified, ST11 was the most common one.

Conclusion

Taken together, our results suggest a capsule/LPS/MLST genotype D/L6/ST11 of *P. multocida* is likely to be strongly associated with swine respiratory disease in China. To further study the genetic characteristics of swine *P. multocida*, we selected 45 strains isolated herein for whole genome sequencing (WGS). Through the strategy and comparative genomic analysis of porcine *P. multocida* genome sequences against *P. multocida* genome sequences from other hosts, we determined the prevalent VFs-associated genes harbored in the swine *P. multocida* epidemic isolates. We also noticed that even though *P. multocida* isolates can be not phylogenetically clustered based on theirs host/disease-symptoms, they can be phylogenetically classified according to their capsule/LPS/MLST genotypes. Our study contributes to understanding the genetic characteristics of *P. multocida* circulating in pigs and other hosts.

Keywords: *Pasteurella multocid*, pigs, genetic characteristic, whole genome sequence, phylogenetic analysis

II-098

Evaluation of the thermo-assisted drying and decontamination system and ozone gas for sanitation of livestock transport vehicles

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Introduction
Pig transportation vehicles are one of the main sources of disease spread among herds, since they move animals with different health statuses over long distances and for different regions. The objective of this work was to validate the thermo-assisted drying and decontamination (TADD) system and the ozone gas in the reduction of total coliforms, as an indicator of contamination, in pig transportation trucks.

Materials and Methods
Surface swabs of ten trucks were performed prior to washing, after washing and disinfection, and after the TADD process and application of ozone gas in the cabin. After the washing and disinfectant application, the outside of the cab and trailer of the trucks were submitted to the TADD system, with an average temperature of 70° C, and 1.5 grams ozone/ hour was released inside the cabin, both for 15 minutes. For collection, sterile bags and sponges were used. To increase the evaluated area, a pool of three samples from each site was performed, comprising 80 samples from each stage and totaling 240 samples analyzed. The samples were seeded in Chromocult® agar, incubated in aerobiose at 37° C for 24 hours, followed by count of colony forming units (CFU) per cm^2. The results were classified as positive or negative growth and the difference between the bacterial growth of each step was evaluated by the chi square test, considering a significant value of $P<0.05$.

Results
Of the 240 samples analyzed, 49 and 39 were positive for total coliforms, before washing and after washing and disinfection, respectively. No sample presented positive growth after the TADD system, statistically differing ($P<0.0001$) from the swabs collected in previous steps. In Brazil, this procedure is being used for the first time.

Conclusion
The disinfection process using TADD system and ozone gas, are efficient in eliminating total coliforms of pig transport trucks.

Keywords: transport biosecurit, disinfection, total coliforms, ozone

Ⅶ-016

Impact of two training implementation plans on post-training competency expression of personnel on farm

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Introduction

Training personnel is an essential part of ensuring competency expression, engaging employees, creating a safe workplace, and developing a workplace that people want to stay. Proper implementation of health and welfare programs by production managers and veterinarians is achieved when integrated with a training program embraced top down by the Human Resources and production management team. In this training implementation case study, a traditional style of training program (Training Program A) was compared against a new training program (Training Program B). Impact on personnel competencies, engagement scores and safety were measured.

Materials and Methods

In Training Program A, farm managers were required to use the system's existing Standard Operating Procedure (SOP) booklet to train staff. Training Program A had a 12-week training guide for each area. Each week of the training had specific tasks that needed to be taught according to the SOP, but teaching method was left up to the manger. Typically, managers would read or talk through the SOP's verbally; and as needed, demonstrate in barn, and provide follow up specific to them or customized to the situation. A check in with each employee was done after 12 weeks.

Results

Training Program B used online learning management software, Pork Avenue Training Portal (delivered by AgriSchool Party Lmt and created by AgCreate Solutions). This program follows a measured "See it, Do it, Teach it" philosophy. Assets consist of videos followed by simulations of desired end skills, graphical SOP's and In Barn Verification Experiences. All learning experiences are assigned on online learning management software which records video and simulation completion as well as completion of the in-barn verification experience to an individual's transcript.

Conclusion

For expressed competency, Training Program A achieved a 59% level of competency. Training Program B achieved over 85% on competency. This was a 26-percentage point increase over Training Program A. Engagement scores were trending upwards at the time of this paper's publication. At the time of presentation, more information will be available. At the time of measurement, 6 months post implementation of the training program, zero injuries had occurred across the whole system.

Keywords: training, competency

VII-033

Truck wash procedures for commercial swine production in Mexico do not meet the industry expectations

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Introduction
Infectious diseases of swine can be transmitted throughout contaminated surfaces of trucks. Therefore the objective of this study was to characterize the procedures to wash and disinfect trucks that are used to move pigs in the main rearing regions of pork production in Mexico. An observational study with convenient sample selection was conducted between January and May 2017. Procedures used to wash and disinfect trucks were evaluated using a survey that included variables related to truck flow within the facility, clean and dirty area separation, equipment, cleaning and disinfection steps, and quality control procedures. Trucks were evaluated before and after washing and disinfection to assess the quality of the process. A descriptive analysis of the data was performed and frequency distributions obtained to estimate trends among the facilities and procedures evaluated.

Materials and Methods
Fifty-nine truck wash sites were evaluated in 13 Mexican states. We estimated that these 59 truck wash sites are associated with the production of 400,000 sows. However, only 44 (74.5%) out of these 59 truck wash sites were considered for this study because the remaining 11 (25.5%) did not meet the minimum requirements to achieve a minimum standard for an appropriate truck wash and disinfection. In 72.7% of the sites organic material was found in the trucks after washing and disinfection. Additionally, in 88.3% of the sites the procedure was not verified after finished. Furthermore, 81.8% of the facilities did not have differentiation between clean and dirty areas. Moreover, a total of 116 trucks were evaluated after washing and disinfection and 72 (62%) did not pass the visual inspection because they contained residues of organic material.

Conclusion
In summary, we found several areas of opportunity to improve washing and disinfection process used for trucks that move pigs in the main rearing regions of pork production in Mexico. Moving pigs in trucks that are not appropriately cleaned and disinfected could increase the risk of transmission of infectious diseases of swine such as PRRS and PED. In addition, the condition of the bodies of the transport units hinders the correct removal of organic matter hence may increase the risk for the farm.

Keywords: truck wash procedures, truck biosecurity

Ⅶ-017

Cross-fostering practices in a farrow-to-finish commercial farm

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Introduction
The objective of this study was to identify cross-fostering (CF) practices in a 1,500 sow farrow-to-finish commercial farm.

Materials and Methods
Pigs [n=1,016; average number of piglets born alive (NBA)=13.0±2.7; average birth weight=1.3±0.32 kg, range 0.45 to 2.42 kg] born within one wk were retrospectively classified according to the wk of lactation when they were CF as 1) non-CF (NCF); 2) CF during the first (CFW1) and 3) second or third (CFW2+) wks of lactation. This was an observational study whereby pigs were managed as per usual practice on the farm.

Results
29.9% of pigs were CF with three CF practices identified. 40.8% of CF pigs were CFW1 (i.e. early); these were born to sows with a higher NBA than NCF pigs (14.6±2.61 and 12.8±2.68, respectively). Hence it is likely that 12.2% of total NBA (i.e. 124 CFW1 pigs out of 1,016 pigs) were CF to reduce variation in litter size. The remaining 59.2% of CF pigs were CFW2+ (i.e. late) and they were, on average, 0.14 kg lighter at birth than NCF pigs. Thus, it is likely that 17.7% of total NBA (i.e. 180 CFW2+ pigs of 1,016 pigs) were CF to standardise body weight within litters. The third practice observed was repeated CF. Up to 27.8% of CF pigs were CF more than once during the lactation period. CFW1 pigs were 4.5 times more likely to be repeatedly CF compared with CFW2+ pigs. It is possible that this was because being initially CF at an early stage in lactation they were more likely to be CF again to balance litters in which piglets died (10.4% of NBA died during lactation).

Conclusion
Although it could be argued that the three CF strategies were particular to this farm, it is likely that similar strategies are employed on many large commercial farms trying to deal with the challenges posed by large litters of piglets with highly variable body weights. As late and repeated CF pose major risks to pig health and welfare alternative strategies such as nurse sows should be employed to manage large litters

Keywords: cross-fostering practices, body weight standardisation, litter equalisation, repeated cross-foste, pigs

VII-004

Impact of runting on colostrum intake, survival chances and development

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Introduction
Runt pigs are intrauterine growth retarded animals characterized by low birth weights (BW). They have higher mortality rates, reduced daily gain and pork quality and increased feed conversion rate. Colostrum intake (CI) is negatively correlated with BW and litter size. For current breeds, a minimum BW of 1.13 kg is needed for normal survival chances. The objective of this study was to investigate the relation between low BW, CI, mortality and development.

Materials and Methods
All piglets from 22 litters were identified at birth and weighed at birth, 24 hours, days 14, 28 and 63. CI was determined (Devillers method). Mortality information (weight, date and reason) was recorded. CI, body weight, average daily gain (ADG) and mortality incidences for low BW piglets (below 1.13 kg) were compared to the others. For statistical analysis, breed and parity were included as block effects and litter size as covariate.

Results
A third of the piglets had a low BW. Their CI was lower per piglet (169 vs 269 g), but not per kg (188 vs 194 g). Their mortality rate was continuously over 4 times higher. Their pre-weaning mortality was 46%, mainly due to low viability and crushing. CI of piglets that died was lower when compared to the survivors irrespective of BW. Although CI per kg of the surviving low BW piglets was comparable to the other piglets, their ADG was always lower resulting in lower weaning (6.27 vs 7.63 kg) and nursery weights (15.66 vs 19.87 kg).

Conclusion
Independent of the underlying cause, failure to consume sufficient colostrum leads to poor survival chances. Low BW clearly predisposes piglets for poor CI. Even when piglets below 1.13 kg consume sufficient colostrum, they still fail to develop like their heavier littermates, indicating that runt pigs are negatively impacted well beyond birth.

Keywords: runt, colostrum intake, survival, development

Ⅶ-039

Use of infra-red thermography to assist control of evaporative cool cells

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In hot climates, which are typical in tropical Asia, the cooling for pigs is a vital part of ensuring that the production, health and welfare of the farmed pigs is optimised. An important tool for providing this cooling is an evaporative cool cell. The evaporative cell works by the hot air moving through damp corrugated paper or alternative water absorbing materials. The heat in the incoming air is transferred into the water on the corrugated surface, resulting in evaporation from the damp surface, thus reducing the air temperature of the air by about 5^{o}C but increasing the relative humidity.

Unfortunately, in practice the management and maintenance of these evaporative cool cells is general poor. The cell is often not sealed properly, and because the evaporative cool cell creates resistance against the incoming air, it is easier for the hot air to enter the building using the unsealed areas, avoiding the cooling effect and creating draughts, all which stress the pigs.

Many evaporative cool cell's water supply systems are not managed and the water is not distributed evenly along the cool cell. This leads to dry streaks of cardboard, where there is no water in the evaporative cell. These dry areas thus provide no cooling effect.

Both of these serious management failings can be easily missed and the cause of a respiratory or feed intake issue misdiagnosed. However, with the reduction in price of commercial infra-red cameras, these management failings can be easily identified by the veterinarian. The camera highlights the distribution of heat over the surface of the evaporative cool cell in a variety of colours. The hotter streaks are easily highlighted. Actual temperatures can be registered. The veterinarian can thus provide a very useful aid to assist the implementation of standard operating procedures.

The picture obtained provides a great teaching tool and point of discussion and can greatly enhance the veterinarian's field report.

Keywords: infra-red, air, cool-cell, temperature, environment

VII-026

Field evaluation study on post vaccination reactions of one-shot Pneumostar SIV® versus a commercial two-shot SIV vaccine in young pigs

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Introduction
Swine influenza (SIV) in commercial farms in Luzon, Philippines has been reported since 2003. While vaccination complemented with strict biosecurity measures are still considered the most effective means of prevention and control, adverse post-vaccination reactions (PVR) to the commercial vaccines is a concern. This study compared post vaccination reactions between a one-shot Pneumostar SIV® (Elanco) versus a two-shot commercial SIV vaccine and was conducted in a 5,000 farrow-finish farm. The two SIV vaccines had the same strains H1N1 and H3N2.

Materials and Methods
A total of 2,648 pigs at 8-12 weeks old, housed separately in 3 houses with 20 pens each containing 45-50 pigs per pen, were randomly assigned in 3 treatments, namely: (1) 1st-shot of SIV+Ceftiofur, (2) SIV 2nd-shot+PRV and (3) one-shot Pneumostar SIV®+PRV. Resting temperatures from 10-16 pigs randomly selected per group were taken and followed at pre-vaccination 0hr, then post-vaccination <1hr, >1hr, 4hr, 8hr, 12hr, 24hr, 48hr while feed intake and mortality were also monitored throughout the observation period.

Results
showed that treatment 1 had the highest number of clinical scores (90% of the subjects), the highest peak temperature (41-42°C) and longest duration of fever response occurred 4-8 hrs post-vaccination at. Pigs in treatment 1 consumed the least amount of allocated feeds at <200 grams. Treatment 2 had 4 mortalities while Treatment 3 showed the least number of clinical scores.

Conclusion
Overall results indicate that one-shot Pneumostar SIV® had the lowest PVR. Although the cause of adverse PVRs was not determined in this trial, the adjuvant can be considered as an important factor. Further investigation on different environmental setting, animal breed and SIV in grouping with other vaccines can be recommended for further evaluation of PVR of commercial SIV vaccines.

Keywords: PVR, SIV vaccine

VII-028

Control of swine influenza A virus endemic persistence in farrow-to-finish herds: insights from a stochastic metapopulation model

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Introduction
Swine Influenza has been shown to persist in an enzootic form in farrow-to-finish farms with recurrent occurrence in successive batches at a similar age. The specific population dynamics of farrow-to-finish pig farms, the immune status of the animals at the infection time and the co-circulation of distinct subtypes and their reassortants leading to consecutive even concomitant infections have been evidenced as factors favouring swIAV persistence within herds. The aim of this study was to evaluate the efficacy of control strategies related to herd management and/or different vaccination schemes using a modelling approach.

Materials and Methods
An event-driven stochastic metapopulation model has been developed to represent the co-circulation of two distinct swIAVs within a typical farrow-to-finish pig herd comprising two subpopulations: breeding sows and growing pigs. Model parameters were estimated in specific experiments. Model outputs were compared to field data to assess the model validity. Different control strategies were evaluated including different vaccination schemes (batch to batch or mass vaccination of the sow herd and vaccination of growing pigs) alone or in association with the export of piglet batches at weaning.

Results
The introduction of one infected gilt in service room led to endemic swIAV within-herd persistence as observed in the field. Although some vaccination schemes (batch-to-batch vaccination) had a beneficial effect in breeding sows by reducing swIAVs persistence within this subpopulation, no vaccination strategies achieved swIAVs fade-out at the within-herd pig population level. The export of consecutive piglets batches at weaning, whatever the vaccination scheme, was found as the most efficient measure facilitating swIAV infection fade-out (HR = 13.7 [8.0 C 23.4]).

Conclusion
Batch-to-batch vaccination of sows, performed before farrowing to induce high antibody levels in piglets, showed limited efficacy in controlling swIAV within-herd persistence alone. Indeed, the higher proportion of piglets with maternal immunity increases swIAV persistence because of longer periods with shedding animals at the batch level. Introducing gaps in the growing pig population and preventing virus transfer between the breeding and the growing part of the herd through increased internal biosecurity are key factors for swIAV control.

Keywords: swine Influenza A viruses (swIAV), transmission dynamics, persistence

VII-037

Current clinical status of *Lawsonia intracellularis* infection in Korea: a lack of acclimatization?

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Introduction
Lawsonia intracellularis, an obligate intracellular bacterium causes porcine intestinal adenomatosis, necrotic enteritis, and proliferative hemorrhagic enteropathy depending on the time of infection. The disease causes enormous economic losses in intensive pig production systems all over the world. There have been reports from Korean pig farms that gilts of high-health-status breeding farms have suffered from *L. intracellularis* induced enteropathies in various forms and economic losses in 3 weeks since they were introduced into new PS farms. Those farms complained a lot about broken gilts and were afraid of the possibility that they could contaminate existing herds. The question is: *L. intracellularis* has been everywhere all the time. Why now? *L. intracellularis* was picked up from environment and/or pigs to pigs; it means it is not the disease of eradication, but of management. The final goal of the *L. intracellularis* enteritis is to suppress the development of clinical symptoms and control growth retardation.

Materials and Methods
Risk assessment was conducted on farms where *L. intracellularis* has caused problems since new gilts were introduced: 1) immune status of the gilts before introduction to PS farms, 2) clinical condition monitoring of high-health-status breeding farms.

Results
The breeding farm was shown to be positive for antibody to *L. intracellularis* but clinical manifestations were very low. However, the gilts at the time of sale were distributed to the PS farms being negative for antibody to *L. intracellularis*. In the absence of full immunity to newly introduced gilts through sufficient acclimatization, they presented clinical signs after transferring into service or pregnant barns. However, existing pigs did not show any clinical abnormality even when the new gilts presented diarrhea or died.

Conclusion
The result suggested that the most important thing is to understand the current disease status of our farm through regular monitoring; when seroconversion occurs or when clinical symptoms occur. To establish preventive measures is followed; vaccination, antibiotic treatment, and acclimatization.

Keywords: *Lawsonia intracellularis,* acclimatization

Ⅳ-004

Age estimation of porcine wounds based on gross evaluation

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Introduction
Age assessment of skin wounds in pigs is crucial and a predominant request for monitoring welfare and in veterinary forensic pathology. A model characterizing gross changes relevant for age assessment of wounds in pigs is non-existing. Therefore, a porcine model of surgically incised wounds healing by second intention was established.

Materials and Methods
In total, 25 pigs were anesthetized before surgical incision of four wounds each. The wounds (n=100) were located on the back and left to heal from 1 h and up to 35 days, at which time the pigs were euthanized. In 14 pigs, biopsies were sampled from two wounds between day 2 and 18. Once a day, the wounds were evaluated for scab formation, epithelialization, wound contraction, swelling and reddening of the edges. All wounds were subjected to gross evaluation until the day of biopsy sampling. Granulation tissue was registered as present or absent in the biopsies and on cross sections of wounds made post mortem. The study and the experimental procedures were approved by the Danish Animal Inspectorate (2013−15−2934−00849).

Results
During the first 24 hours, all wounds were characterized by hemorrhage and the formation of a clot and a scab. From day 2 to 5 the majority of wounds were surrounded by swollen and red edges. Granulation tissue was observed from day 5 to 35 (40 out of 40 wounds, 100%). Contraction lines and epithelialization were seen from day 6 (5 out of 36 wounds, 14%) and day 7 (2 out of 32 wounds, 6%), respectively. However, due to the scab covering the wounds, both contraction lines and new epithelium might already have been present at an earlier time.

Conclusion
In conclusion, the presence of granulation tissue can be used to determine if wounds are at least 5 days old. Other gross characteristics were too variable in order to estimate the age of wounds.

Keywords: age of wounds, pig, forensic pathology, wound

IV-034

Risk and protective factors for health, welfare and performance in sows and piglets in 8 EU countries. Part 1: focus on health-related performance parameters

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Introduction
Production diseases are often of multi-factorial origin in which environment (housing, nutrition and management) and pathogenic challenges show complex interactions. The aim of this study was to identify risk factors regarding health, welfare and performance in sows and piglets, in diverse systems using data from 8 EU countries (Germany, the Netherlands, Finland, Spain, Belgium, Italy, Portugal, Denmark).

Materials and Methods
A specific questionnaire was developed for sows and piglets covering farm management, vaccinations and housing. 169 farms were visited once between February and October 2016.
From 156 farms, production data such as alive born piglets per litter (ABP), dead born piglets per litter (DBP), preweaning mortality rate (PM) and weaned piglets per litter (WP) were collected covering 2014 and 2015.
Linear models were employed to identify risk factors at a supranational level. For each selected question (n:66), all associations between this and the studied performance parameter were first tested univariably. Variables with *P*-values of < 0.2/8 (Bonferroni correction for multiple testing) were retained for further analysis in a multivariable model. In these final models, associations were considered significant if *P*< 0.00625.

Results
Various risk and protective factors were identified per parameter tested. The level of importance -to a farmer- for the genetic selection for resistance against diseases had a negative effect on ABP, DBP, PM and WP. The presence of a cooling system in the gestation unit positively affected ABP while an older fattening unit facility negatively affected ABP. PRRS sow vaccinations were associated with a lower DBP. WP was positively associated with a more frequent feeding of sows in the farrowing unit (WP), and with a lower time interval after insemination before the sows were placed in group housing (WP).

Conclusion
Over all countries, various factors related to applying good management practices, investing on genetic selection for robustness (resistance against diseases) and improving housing had a significant protective effect on various health-related performance parameters. Multivariable statistical analyses at supranational level ensure a better understanding of multifactorial production diseases in pigs.

Keywords: production diseases, sow management, porcine health

Ⅳ-065

Influence of different farrowing and weaning systems on the welfare and health of weaner pigs

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Introduction
In modern pig husbandry, animal welfare is an important factor for raising healthy animals. In this study effects of early socialization in farrowing units and effects of mixing pigs at different ages on their welfare and health were examined.

Materials and Methods
Piglets were raised either in single-litter-systems with conventional farrowing crates (FC) or free-movement-pens (FMP) or a multi-litter system with group-housing (GH). There were additionally three different postweaning systems: a standard system with mixing and regrouping at weaning (control group CG), a system where the weaners were left in their farrowing system (weaning-in-farrowing unit WiFu) and a wean-to-finish (WetoFi) system with more space availability and concrete slatted flooring. Eight consecutive batches were performed, where piglets were tail-docked or undocked batch wise. Scorings for claw health, skin lesions, lameness, diarrhea and coughing bouts were performed every two weeks during the weaning period (T1, T3, T5), tail lesions were scored weekly (T1 – T5).

Results
At T1 GH-piglets had less skin lesions than the FC- or FMP-piglets. From T1 to T5, skin lesions decreased in the CG and the WetoFi-group in contrast to the WiFu-group. In the WetoFi-group significantly more claw lesions developed at T1 than in the other groups, especially at the solear horn and the coronet. From T1 to T5, claw lesions decreased again and were less severe in all groups. Tail lesions increased from T1 to T5 and were more severe in all weaning-systems, especially in undocked CG-pigs. At T5, GH-piglets showed more severe tail lesions than FC- or FMP-piglets.

Conclusion
Less skin lesions in GH-piglets indicate a positive benefit of early socialization. Development of claw lesions shortly after weaning depended on the occurrence of agonistic behavior and on floor conditions. Leaving the piglets in their farrowing crate seems to be the least stressful kind of weaning. More frequent and severe tail lesions at the end of weaning may be a result of less space availability and insufficient enrichment in the pens.

Keywords: farrowing/weaning systems, animal welfare

IV-059

Effect of feeding method on the behavior, physiology and reproductive performance of group-housed pregnant sows

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Introduction
In group feeding systems, gestational sows are generally chronically stressed because of food restriction and poor environmental conditions. The objective of this study was to investigate whether providing pellet-type feed in the straw litter rather than in a trough improves sow welfare.

Materials and Methods
One hundred and thirty-two sows (44 Large White, 44 Landrace, 44 Duroc) of parity 5 to 6 were assigned to the DS group (pellet-type feed artificially dropped into straw litter evenly) or the CS group (pellet-type feed put into a trough). From 0600 to 1800-h on the Monday of the 1st, 3rd, 7th, 11th, and 15th weeks of pregnancy, grooming, sham-chewing, feeding, water drinking, bar-biting, fighting, and manipulation (housing pen, straw, companion) behaviors were recorded from video footage. On the Friday of these weeks, blood samples were collected 30-min before and 1-h after feeding, and the concentrations of serum C reactive protein, hemoglobin and glucose were measured. The incidence of disease and degree of injury to the head, ear, neck, shoulder, loin, and back of the sows were recorded in these weeks. Postpartum reproductive performance-related indicators were recorded.

Results
The results showed that DS reduced sham-chewing, bar-biting, fighting, and drinking behaviors (all $P< 0.01$), increased manipulation (housing pen, straw, companion) behaviors (all $P< 0.01$), and had no effect on grooming behavior ($P> 0.05$). DS also increased serum C reactive protein, hemoglobin, and glucose concentrations (all $P= 0.017$), but had no effect on salivary cortisol following ACTH stimulation ($P> 0.05$). Feeding method had no effect on morbidity ($P> 0.05$), but affected the degree of injury of the head, ear, neck, shoulder, loin and back (all $P< 0.001$ except neck, $P= 0.04$). Feeding method also affected farrowing duration and interval and the number of weak piglets (all $P< 0.05$), but none of the other reproductive performance measures ($P> 0.05$).

Conclusion
In conclusion, pellet-type feed added to the straw litter enabled foraging behavior and improved the welfare of group-housed pregnant sows, but did not eliminate chronic stress.

Keywords: behaviors, feeding methods, physiology, pregnant sows, reproductive performance

Ⅳ-008

A comparison of immunocastrated pigs versus physically castrated pigs and entire males: meta-analysis of most relevant parameters for pig producers

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Introduction
The study aimed to compare pigs immunologically castrated (IC) with Improvac® versus physically castrated (PC) or entire male (EM) pigs, using meta-analysis techniques.

Materials and Methods
Comparative studies without the feed additive ractopamine were considered for analyses of mean differences in growth performance and carcass data, overall and stratified according to the time between 2nd Improvac® dose and slaughter. Risk of boar taint was assessed by comparing the number of pigs exceeding the established consumer thresholds of detection (ToD) for skatole (0.2 µg/g fat) and androstenone (0.5 µg/g fat).

Results
Compared to PC pigs average daily gain (ADG) of IC pigs is 26.3 g higher with 223 g less feed consumed per kg of body weight gained. Live weight at slaughter is >2 kg higher, whereas hot carcass weight (HCW) is numerically but not statistically significantly lower. On average, percentage lean meat is 1.3% units higher and backfat 2 mm thinner. A shorter time between 2nd injection and slaughter (<4.5 weeks) maximizes the ADG, whereas increasing the interval has the effect of increasing carcass fat, although IC pigs remain significantly leaner than PC pigs. Compared to EM pigs, ADG in IC pigs is 59.4 g higher. They have a higher live weight at slaughter (+3.3 kg) and HCW (+1.2 kg), less lean meat and more backfat. Time between 2nd vaccination and slaughter only has a small impact on ADG, but the increase in live weight and backfat in IC pigs becomes more distinct at ≥4.5 weeks interval. No statistically significant differences were observed between the number of IC pigs and PC pigs exceeding ToD for skatole and androstenone whereas significantly more EM pigs exceeded these ToDs.

Conclusion
Meta-analyses of studies comparing IC pigs with PC or EM pigs confirmed previously published advantages of IC pigs from the perspective of the producers, also demonstrating the option to tailor pork meat to different market situations by varying the time between 2nd vaccination and slaughter while effectively avoiding boar taint.

Keywords: immunocastration, pig producer, meta-analysis, Improvac

IV-022

Revitalizing weak new born piglets with glucose and heating

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Introduction
When litters of newborn piglets are observed first time after birth, some piglets are weak and do not fight for a teat. Some of these piglets are cold and hypoglycemic. A previous investigation indicated that piglets with a rectal temperature below 37.5°C and blood glucose below 2.8 mmol/L were cold and hypoglycemic. (Thorup and Diness, IPVS 2016). It was investigated what it takes to restitute these piglets.

Materials and Methods
This trial was performed under permit 2017-15-0201-01220. Piglets born during the night were weighed in the morning. From piglets weighing below 1 kg, 41 piglets with a blood glucose level below 2.8 mmol/L were selected for the trial (Accu-chek Aviva Nano, Roche Diagnostics). Piglets were randomized to receive a solution containing 0.55, 1 or 2 g glucose orally, and then placed in an insulated container at 32-36°C for four hours. Rectal temperature and blood glucose were measured every 30 minutes. After 4 hours, the piglets were returned to the farrowing unit. Survival was registered after 7 days.

Results
Rectal temperature increased rapidly the first hour after treatment. 0.55 and 1 g glucose orally normalized blood glucose level in the piglets. Two gram glucose increased blood glucose to levels of hyperglycemia. One hour after receiving 0.55 g glucose, the level of blood glucose had returned below 2.8 mmol/L in many of the piglets. Blood glucose returned to basal levels after 2.5 hours in piglets given 1 or 2 g glucose. Three of 15 piglets weighing between 450 and 650 g were still alive 7 days after the trial. All 26 piglets weighing between 690 and 1000 g were alive at 7 days of age.

Conclusion
Cold and hypoglycemic piglets can be revitalized with 1 g glucose orally and 1 hour at a temperature of 36°C.

Keywords: newborn, piglets, weak, glucose, hypoglycemia

Ⅳ-020

Plasma and milk concentrations of tiamulin hydrogen fumarate when administration via drinking water to periparturient sows

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Introduction

Sows receiving water-soluble tiamulin hydrogen fumarate (THF) per os at 20mg/kg body weight excreted tiamulin in milk at concentrations up to 4.9 µg/mL, a therapeutic concentration against respiratory and systemic pathogens such as *Mycoplasma* spp.*, Streptococcus suis, Hemophilus parasuis*. This study's objective is to determine lactating sows' THF plasma and milk concentration during and after drinking water treatment at three different THF concentrations.

Materials and Methods

Thirty-two periparturient multiparous sows were randomly assigned to one of four THF concentrations treatment groups. Control (C) 0 ppm, Low (L) 60 ppm, Medium (M) 120 ppm, or High (H) 180 ppm. THF treatment was for 5 days (2 days before, day of, 2 days after parturition). Plasma and milk collection continued for 4 days after THF treatment. Daily water consumption was recorded. Stock medication mixture, drinking water, plasma, and milk were collected daily and frozen until analyzed using standard liquid chromatography methods.

Results

Actual drinking water THF concentrations were C: 0PPM, L: 81 PPM, M: 89 PPM, and H: 169 PPM. Water intake was not different across treatment ($P>0.17$). THF dose was based on an estimated sow body weight (bw) of 250 kg. The average THF dose was C: 0 mg/kg bw, L: 7.6 mg/kg bw, M: 8.2 mg/kg bw, H: 19.7 mg/kg bw. Average plasma THF concentrations were C: 0 ng/mL, L: 14.3 ng/mL, M: 9.0 ng/ml, H: 23.9 ng/mL. Average milk THF concentrations were C: 0 ng/mL, L: 173 ng/mL, M: 192 ng/mL, H: 494 ng/mL. Three days following withdrawal of THF, milk and plasma THF concentrations were not significantly different from zero ($P<0.05$).

Conclusion

THF is concentrated in the sow's milk 20 times over plasma concentrations in a dose dependent manner. The treatment with THF to lactating sows' drinking water had no effect on water intake. Veterinarians can utilize THF in different doses to deliver a known amount of THF through lactating sows milk.

Keywords: swine, pharmacology, sow milk, pharmacokinetics, tiamulin

IV-057

Pharmacokinetics of ketoprofen in nursing piglets when compounded with iron dextran prior to injection

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Introduction
The Canadian Code of Practice for Care and Handling of Pigs states that as a requirement, pigs must receive analgesia to control post-procedural pain. In Canada, options are limited to the use of non-steroidal anti-inflammatory drugs (NSAIDs) for analgesia. Piglets also receive iron dextran (ID) to prevent anemia as standard practice in North America, and there is evidence that some producers combine ID with NSAIDs, to administer as a single injection, and to minimize piglet handling and labor. The practice of compounding drugs is currently legal in Canada with appropriate justification. The objective of this study was to determine if there was any difference in the bioavailability or pharmacokinetic parameters of ketoprofen when mixed with iron dextran prior to administration, compared to ketoprofen given alone.

Materials and Methods
Commercial piglets, 3-4 days of age (9 male, 9 female), were enrolled and individually housed, fed and monitored. Piglets were administered 1 of 3 treatments via intramuscular injection: (a) ketoprofen (Anafen®, Merial Canada), (b) ketoprofen mixed with ID (Dexafer 200®, Vetoquinol), or (c) ID alone. Fifteen serial blood samples were collected from each piglet via indwelling jugular catheters, and the plasma was analyzed for S- and R- ketoprofen enantiomer levels by mass spectrometry.

Results
Standard pharmacokinetic (PK) analyses were completed for R- and S-ketoprofen enantiomers and comparisons of parameter means (t-test) between treatments (a) and (b) (± SD) showed that there was no difference for S-ketoprofen: Cmax (7631.25 ± 378.43 and 6665.00 ± 261.73, respectively, $P = 0.054$), AUC_{last} (35600.18 ± 5620.37 and 25636.10 ± 2453.69 respectively, $P = 0.127$) or $AUC_{0\text{-infinity}}$ (35869.66 ± 5673.94 and 26151.97 ± 2432.94 respectively, $P = 0.138$).

Conclusion
The same PK parameters for R-ketoprofen were not different (all $P > 0.05$). The lack of statistical difference in the PK parameters supports that the bioavailability of ketoprofen is not altered by the process of compounding with the ID. However, given the small sample size and potential for biological significance, pain control efficacy studies are needed before any further recommendations for the continued practice of compounding these 2 drugs can be made.

Keywords: bioavailability, ketoprofen, iron dextran, analgesia, castration

IV-017

Two step dilution

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Introduction

Artificial insemination (AI) is the most common method for sow fecundation nowadays. More than 90% of swine AI worldwide are carried out with extended liquid semen stored up to 5 days. The temperature during the preparation of the extended semen samples is one critical point. Sudden decrease in temperature is associated with thermotropic phase transition of the sperm membrane lipids. Separation of lipid phase causes alterations of membrane proteins and its permeability, leading to a leakage of cations and enzymes, impairing its viability. During the semen processing, temperature is controlled so semen is not cooled below 16-17ºC.

Materials and Methods

Boar studs produce semen extended samples either by one-step or two-step dilution. In the first, the ejaculate is collected in a pre-warmed recipient and added to a final dilution with pre-warmed (30-33ºC) extender. In the latter, the ejaculate is first diluted 1:1 with pre-warmed extender (30-33ºC) prior to a final dilution with either pre-warmed or room temperature extender. Pre-warming and cooling of large volumes of extender increases time of production and costs. Different AI practices may cause differences on semen quality between AI centers.

Results

Previous studies about the effect of temperature of the dilution on the quality of semen have found no significant differences on the parameters studied. However, a recent study comparing the different two step dilution techniques found a negative effect in the parameters of sperm. Nevertheless, there is no proof that these differences result in impaired results at farm.

Conclusion

The present study compares two-step dilution isothermic and hypothermic on a field experiment. The experiment also evaluates the effect in the offspring derived from extended semen under these conditions.

Keywords: temperature, sperm, artificial insemination

IV-037

Andrological evaluation in high genetic merit boars from maternal and terminal cores lines

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Introduction
Boars of different genotypes have different growth rates and muscle tissue deposition, leading to differences in sexual maturation and the inappropriate culling of high merit boars due to low quality semen or infertility at selection. This work was carried out to compare body and testicular development during the pre-pubertal phase, reproductive and seminal characteristics during puberty between two different strains.

Materials and Methods
Males from two pure breeds used in the crossing of female lines (FL, n=37) and two lines of terminator cores (TL, n=33) were evaluated. Body weight and testicular measurements were taken in the first, third, and 15th weeks of life, reproductive characteristics were evaluated starting 150 days of age, and seminal parameters were collected weekly from the 24th until the 30th weeks of age. Data were analyzed as a randomized complete design using the general linear model (GLM) procedure of SAS (SAS Institute Inc., Cary, NC). Least square means were compared using the Student *T* test with $P< 0.05$ of significance.

Results
TL males presented greater body weights and testicular measures ($P< 0.01$) compared to their FL counterparts. Moreover, 97% of the TL males were trained, while 83% of the FL males were unable to mount the dummy. Genotype did not affect the seminal characteristics evaluated: volume, concentration, number of total spermatozoa, number of viable spermatozoa and motility. The percentage of normal spermatozoa in the morphological evaluation was higher in TL boars ($P< 0.05$). Even though parameters of sperm kinetics, such as DSL, VSL and STR were higher in FL boars ($P< 0,05$), greater percentage of tail defects was observed in FL boars in all samples collected ($P< 0.05$). TL males showed higher precocity and were approved in the seminal morphology evaluation at 185.5 days compared to FL males that were approved later than 201.5 days.

Conclusion
Since there are differences in growth development and sexual maturation between genotypes, knowing the andrological parameters of each genotype before and after puberty will help to establish specific high merit boars' classification or declassification criteria, without the earlier and improper cull of good males.

Keywords: genotypes, semen quality, selection, precocity

Ⅰ-070

Identification of porcine epidemic diarrhea virus variant with a large spike gene deletion from a clinical swine sample in the USA

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Introduction
Two genetically different PEDV strains have been identified in the USA: US non-S INDEL and S INDEL PEDVs. A nucleocapsid or membrane gene-based real-time RT-PCR (rRT-PCR) is generally used for the screening detection of PEDV from clinical specimens. A spike gene-based multiplex rRT-PCR can be further used to differentiate non-S INDEL from S INDEL PEDV strains. Here we present a case report of identifying a PEDV variant harboring large-spike-gene deletion from a clinical swine sample after observing unusual results by nucleocapsid and spike gene-based rRT-PCRs.

Materials and Methods
In February 2017, a rectal swab collected from a sow farm in Oklahoma, USA was positive for PEDV by nucleocaspid gene-based rRT-PCR with CT 15.5; however, spike gene-based differential rRT-PCR revealed this sample was negative for S INDEL PEDV (CT>40) but was weak positive for non-S INDEL PEDV (CT 36.8). It is unusual to have such a big CT difference between two PCRs. To investigate the reasons for this observation, the sample was sequenced using next-generation sequencing technology. The detected PEDV (USA/OK10240-8/2017) had whole genome sequence of 27,438 nucleotides. Phylogenetic analyses based on the whole genome and the spike gene indicated the OK10240-8 strain belongs to the US non-S INDEL cluster. However, compared to non-S INDEL PEDV strains, the OK10240-8 PEDV had a large continuous deletion of 600-nt (200-aa) in the spike gene/protein (nt Δ91-690; aa Δ31-230). A gel-based RT-PCR was developed to differentiate the OK10240-8 PEDV from non-S INDEL PEDV.

Results
Twenty more samples collected from the same farm all contained non-S INDEL PEDV with none containing OK10240-8-like PEDV, indicating the prevalence of OK10240-8-like PEDV may be very low. Virus isolation attempts on the sample in Vero cells were unsuccessful. The remaining sample was orally inoculated into two 10-day-old PEDV negative piglets but did not result in active infection.

Conclusion
This study is the first report of detecting PEDV bearing a large-spike-gene-deletion in clinical swine samples in the USA. The pathogenicity of this PEDV variant remains to be determined. Additional molecular epidemiological studies are needed to monitor the emergence of novel PEDV variants and determine their prevalence levels in US swine.

Keywords: porcine epidemic diarrhea, variant, large deletion in S

I -106

Two trafficking signaling motifs at the end of the spike protein of porcine epidemic diarrhea virus are virulence determinants in pigs

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Introduction
Porcine epidemic diarrhea virus (PEDV) causes high mortality in neonatal pigs, but viral genetic factors contributing to pathogenicity are not well identified. A premature terminated (ΔEVFEKVHVQ) spike (S) protein was identified in the 120th and higher passage levels of the cell culture-attenuated original US PEDV strain PC22A. Similar S proteins were also reported for several Vero cell-attenuated PEDV strains or mild field variants. Without affecting any known virus neutralizing epitopes, these PEDV variants lose partial YXXF and/or KXHXX motifs. These two motifs are intracellular trafficking signals critical for retaining S proteins in PEDV assembly sites, the ER-Golgi intermediate compartments.

Materials and Methods
To investigate whether these motifs are virulence determinants, we generated three recombinant viruses with the single or double motif-deletions by introducing stop codons or an amino acid substitution into the infectious clone of virulent PC22A strain (icPC22A): 1) icPC22A-S2Δ10aa (ΔYEVFEKVHVQ); 2) icPC22A-S2Δ5aa (ΔKVHVQ); and 3) icPC22A-Y1378A (inactivated motif AEVF). We orally inoculated (100 PFU/pig) 5-day-old gnotobiotic pigs (n=4-5 per group) with each mutant, and virulent icPC22A. Two pigs were mock inoculated with cell culture medium.

Results
Within 52 hours post-inoculation (hpi), all PEDV-infected piglets developed diarrhea. However, piglets inoculated with icPC22A-S2Δ10aa had a significantly lower rate (50%) of severe diarrhea, shed significantly lower titers of infectious virus in feces, and displayed milder intestinal villous atrophy than pigs in the other three virus-inoculated groups. In Vero cells, icPC22A-S2Δ10aa and icPC22A-Y1378A replicated to significantly lower titers but formed significantly larger plaques than icPC22A and icPC22A-S2Δ5aa. Immunofluorescent staining of S proteins of PEDV-infected Vero cells (8 hpi) showed that the amounts of S proteins on the PEDV-infected cell surface were lower for icPC22A and icPC22A-S2Δ5aa than for icPC22A-Y1378A and icPC22A-S2Δ10aa, indicative of the defective in internalization of the S proteins from cell surface of icPC22A-Y1378A and icPC22A-S2Δ10aa.

Conclusion
These results suggest that the two motifs at the end of the S protein are virulence determinants of PEDV in pigs. The loss of the motifs likely results in more S proteins on the cell surface, triggering more cell-to-cell membrane fusion to form larger syncytia, but fewer infectious virus particles assembled.

Keywords: porcine epidemic diarrhea, PED, spike, signaling motif

I -117

Characterization of the immune response against porcine hemagglutinating encephalomyelitis virus in grow-finisher pigs

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Introduction

Porcine hemagglutinating encephalomyelitis virus (PHEV) is the only known neurotropic coronavirus affecting pigs, being a potential threat to high health gilts herds. PHEV can infect naïve pigs of any age but clinical disease is age-dependent. In growing pigs and adults, PHEV infection is subclinical, but acute outbreaks of vomiting and wasting syndrome, and encephalomyelitis may be seen in neonatal pigs born form naïve sows, with mortality rates reaching 100%.

Materials and Methods

In this study, we characterized the viral dynamics and immune response in PHEV in 7-week-old pigs (n = 12) over the course of infection. A mock inoculated (culture media) group (n = 12) was included as negative control. Viral shedding was evaluated daily on pen-based (6 pens, 2 pigs per pen) oral fluids and feces throughout the study. Serum samples were collected at -7, 0, 3, 7, 10, 14, 17, 21, 28, 35 and 42 days post-inoculation (DPI) to evaluate both viremia and humoral immune response by real-time RT-PCR and a protein-based indirect ELISA, respectively. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood on 0, 3, 7, 10, 14 and 21 DPI to evaluate cellular immune response.

Results

Mild neurological signs including tremor and generalized muscle fasciculation were reported in 2/12 pigs at 4-6 DPI. Virus shedding was consistently detected by real-time RT-PCR assay in pen oral fluids (DPI 1-28) and feces (DPI 1-10). Viremia was not detected throughout the observation period. Isotype-specific antibody responses in serum showed a strong IgM response at 7 DPI that declined quickly after 14 DPI. Strong IgA and IgG responses were detected by DPI 10 and declined gradually after 28 DPI. Flow cytometry analysis revealed an increase on both monocytes (DPI 10) and cytotoxic T cell (DPI 21) populations in response to PHEV infection. We postulate that the T-cell response is the key to virus clearance.

Conclusion

This study sheds light in the humoral and cellular immune responses, and viral shedding patterns in an experimental setting. This information will be useful in detecting and monitoring PHEV infections.

Keywords: porcine hemagglutina, immune response, grow-finisher pigs

I -234

Porcine hemagglutinating encephalomyelitis coronavirus in Argentina. A serological survey on farms of high, medium and low biosecurity

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Introduction
Porcine hemagglutinating encephalomyelitis coronavirus virus (PHEV) is a neurotropic virus that affects pigs ≤ 3 week of age. The clinical disease is characterized by neurologic and/or digestive disorders associated with high mortality, reaching 100% mortality in naive farms. The infection has been reported in all of the major pig-raising countries in Europe, Asia, and North America, where the infection appears to be endemic and virus circulation seems to undergo subclinical. Thus far, only Argentina and China have reported clinical outbreaks. Serological tests available for PHEV included: IFAT, HI, VN, ELISA and lateral flow immunochromatographic assay. The aim of this study was to evaluate the seroprevalence of PHEV on farms of high (HB), medium (MB) and low biosecurity (LB) in Argentina without clinical sings suggestive of PHEV infection.

Materials and Methods
Seventeen farms were selected and clustered by their biosecurity status as follow: 7 with HB, 5 with MB, and 5 with LB. A total of 961 samples from 14 breeding herds and 3 farrow-to-finish farms were evaluated. Blood samples were collected from 30 randomly selected gilts, sows or growing/fattener pigs. The association between the biosecurity categories, farm size, positive sows/gilt/fatteners in the herd and within-herd prevalence was analyzed by Chi-square test and One-way analysis of the mean (ANOVA). The presence of PHEV specific antibodies was evaluated by an indirect ELISA based on the amino terminal portion of the PHEV spike protein (S1).

Results
The overall seroprevalence was 41.62 % (CI: ± 3.12). The percentage of positive farms was 100%, 80% and 60% for HB, MB and LB farms, respectively. The percentage of positive sows was 45.71% (CI: ±4.41), gilts 43.57 % (CI: ±8.21) and grower/fattener pigs 34.74% (CI: ±5.13). Amongst positive farms, the within herd prevalence varied from 12.5% to 86.6% for sows, 25% to 85.7% for gilts and 3.7% to 90% for grower/fattener pigs. No statistical differences were observed on seroprevalence by age category or biosecurity status. The presence of antibodies in grower/finisher suggested that colostral antibodies may persist for more than 6 weeks or animals were subclinically infected during the grower-finisher stage.

Conclusion
This survey demonstrates that PHEV is widespread and undergoes subclinical in Argentina farms.

Keywords: porcine hemagglutinating encephalomyelitis virus, seroprevalence, biosecurity

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