Importance of domestic pigs as a reservoir for autochthonous hepatitis E in France

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6th International Symposium on Emerging and Re-emerging Pig Diseases

Barcelona 12-15 June, 2011

Proceedings

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Very importantly, in order to guarantee the scientific and technical quality of the information presented in the Symposium, a panel of reviewers is needed. Therefore, we wish to warmly thank the following reviewers for reading and scoring the abstracts and assisting with the organization of the program:

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UNDER THE AUSPICES OF

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LOCATIONS OF INTERNATIONAL SYMPOSIUM ON EMERGING AND RE-EMERGING PIG DISEASES CONGRESSES

1991 Minneapolis (United States)
1992 Minneapolis (United States)
1995 Copenhagen (Denmark)
1999 Ploufragan (France)
2003 Rome (Italy)
2007 Krakow (Poland)
2011 Barcelona (Spain)

PREFACE

On behalf of the 6th International Symposium on Emerging and Re-emerging Pig Diseases Organizing Committee, it is my great pleasure to welcome you to Barcelona, Spain. As in previous editions -starting in Minneapolis (USA) in 1991- the efforts of this International Symposium have been to bring together scientists and veterinarians from various disciplines working around the world in the passionate field of novel and economically significant diseases in swine.

Across its history, this International Symposium had different names and included different scientific topics. At first, the focus was on Aujeszky's disease (AD) eradication but in the last two editions the focus shifted to porcine reproductive and respiratory syndrome (PRRS), swine influenza (SI) and postweaning multisystemic wasting syndrome (PMWS). PRRS and PMWS are still two of the most relevant pig diseases worldwide and, in consequence, they will continue being the core of this Symposium. But SI has not been forgotten. The emergence of the novel pandemic human influenza virus A(H1N1) in 2009 awoken the fear of a global threat for human health. Although the evolution of the influenza pandemic has not accomplished the most dramatic predictions, this episode reinforced the notion that pigs may play a potential role in the generation of new influenza viruses. Therefore, SI is again another of the issues of the present Symposium. In addition, and most importantly, the 2011 Symposium aims at fostering the coherence of its essence: emerging and re-emerging pig diseases. Importantly, the number of abstract submissions for this Symposium has been the highest ever, evolving finally to 11 lectures, 35 oral communications and 256 posters all together. We are sure that the most recent and relevant information on emerging and re-emerging diseases of pigs useful for veterinarians and scientists will be presented and discussed during the Symposium.

Last but not least, you are in Barcelona these days. Spain is a magnificent and beautiful country and tourist/vacation possibilities are almost endless. Barcelona is a cosmopolitan city in which you will feel at home. You can choose from culture to environment and nature, from sea to mountains, from exhibitions to partying… you will surely meet up with your match. Discover, taste, experience and enjoy!

Joaquim Segalés
Chair of the 6th International Symposium on Emerging and Re-emerging Pig Diseases Organizing Committee

PROGRAM SCHEDULE

Sunday 12 June 2011	Monday 13 June 2011	Tuesday 14 June 2011	Wednesday 15 June 2011
	08:30h-09:30h **Key note Lecture** **Porcine circovirus diseases** Poul Baekbo	08:30h-09:30h **Key note Lecture** **Porcine reproductive and respiratory syndrome virus** Hans Nauwynck / Raymond Rowland	08:30h-09:30h **Key note Lecture** **Influenza viruses in pigs** Amy Vincent
	09:30h-10:15h **Oral Communications**	09:30h-10:15h **Oral Communications**	09:30h-10:15h **Oral Communications**
	10:15h-11:00h ***Coffee-break & Posters***		***10:15h-12:00h*** ***Coffee-break & Posters*** 10:30h-12:00h **Satellite Symposium** MERIAL
	11:00h-12:30h **Oral Communications**	11:00h-12:30h **Oral Communications**	12:00h-13:30h **Oral Communications**
	12:30h-13:45h ***Lunch***		13:30h-14:00h **FINAL CONCLUSIONS AND CLOSURE**
13:30h-15:30h **Registration**	13:45h-14:45h **Poster session**	13:45h-14:45h **Poster session**	
15:00h **Opening**	14:45h-15:15h **Emerging viruses** Xiang-Jin Meng	14:45h-15:15h **Foot and mouth disease** Emilio León	
15:30h-17:30h **Welcome Roundtable** **One world-one health:** **The threat of emerging diseases** Thomas Blaha R. Thanawongnuwech John Deen	15:15h-15:45h **Torque teno sus virus** Tuija Kekarainen	15:15h-15:45h **African swine fever** J.M. Sánchez-Vizcaíno	
	15:45h-16:45h **Oral communications**	15:45h-16:45h **Oral communications**	
18:00h-19:30h **Get together**	17:00h-18:30h **Satellite Symposia** Boehringer Ingelheim · Pfizer Animal Health	17:00h-18:30h **Satellite Symposia** HIPRA The Reference in Prevention for Animal Health · Intervet Schering-Plough Animal Health	
		20:00h-23:00h **Farewell Dinner MNAC**	

SCIENTIFIC PROGRAM

Sunday, 12th June

13.30 Registration

15:00 **Opening**

15.30-17.30 WELCOME ROUNDTABLE *– Auditorio*

One world, one health: the threat of emerging diseases
Chairman: **Robert Morrison**
Speakers**: Thomas Blaha**
John Deen
Roongroje Thanawongnuwech

18.00-19.30 Get together *– Swimming Pool*

Monday, 13th June

08.30-09.30 KEY NOTE LECTURE: *– Auditorio*

Porcine circovirus diseases
Chairman: **Gordon Allan**
Speaker: **Poul Baekbo**

09.30-10.15 ORAL COMMUNICATIONS (O.01 - O.03) *– Auditorio*

Chairman: **Gordon Allan**

10.15-10.45 **Coffee break** *– Exhibition Area – Level -1*

10.15-11.00 POSTER SESSION *– Level -1*

11.00-12.30 ORAL COMMUNICATIONS (O.04 - 0.09) *– Auditorio*

Chairman: **John Harding**

12.30-13.45 **Lunch** *– Exhibition Area – Level -1*

13.45-14.45 POSTER SESSION *– Level -1*

14.45-16.45 EMERGING VIRAL INFECTIONS *– Auditorio*

Chairmen: **Joaquim Segalés**
Satoshi Otake

14.45-15.15 EMERGING VIRUSES
Speaker: **Xiang-Jin Meng**

15.15-15.45 TORQUE TENO SUS VIRUS
Speaker: **Tuija Kekarainen**

15.45-16.45 ORAL COMMUNICATIONS (O.10 - O.13) *– Auditorio*

17.00-18.30 SATELLITE SYMPOSIA

Boehringer Ingelheim *– Room F*
Pfizer *– Room J*

Tuesday, 14th June

08.30-09.30 KEY NOTE LECTURE: *– Auditorio*

Porcine reproductive and respiratory syndrome virus
Chairman: **Enric Mateu**
Speakers: **Hans Nauwynck**
Raymond Rowland

09.30-10.15 ORAL COMMUNICATIONS (O.14 - O-16) *– Auditorio*

Chairman: **Enric Mateu**

10.15-10.45 **Coffee break** *– Exhibition Area – Level -1*

10.15-11.00 POSTER SESSION *– Level -1*

11.00-12.30 ORAL COMMUNICATIONS (O.17 - O.22) *– Auditorio*

Chairman: **Tomasz Stadejek**

12.30-13.45 **Lunch** *– Exhibition Area – Level -1*

13.45-14.45 POSTER SESSION *– Level -1*

14.45-16.45 RE-EMERGING VIRUSES *– Auditorio*

Chairmen: **Zygmunt Pejsak**
Paolo Martelli

14.45-15.15 FOOT AND MOUTH DISEASE
Speaker: **Emilio León**

15.15-15.45 AFRICAN SWINE FEVER
Speaker: **J.M. Sánchez-Vizcaino**

15.45-16.45 ORAL COMMUNICATIONS (O.23 - O.26)

17.00-18.30 SATELLITE SYMPOSIA

Hipra *– Room F*
Intervet *– Room J*

20.00-23.00 **FAREWELL DINNER** - MNAC (Museu Nacional d'Art de Catalunya, National Museum of Art of Catalonia)

Wednesday, 15th June

08.30-09.30 KEY NOTE LECTURE *– Auditorio*

Influenza viruses in pigs
Chairman: **Janice Ciacci-Zanella**
Speaker: **Amy Vincent**

09.30-10.15 ORAL COMMUNICATIONS (O.27 - O.29) *– Auditorio*

Chairman: **Janice Ciacci-Zanella**

10.15-10.45 **Coffee break** *– Exhibition Area – Level -1*

10.15-12.00 POSTER SESSION *– Level -1*

10.30-12.00 SATELLITE SYMPOSIUM

Merial *– Room F*

12.00-13.30 ORAL COMMUNICATIONS (O.30 - O.35) *– Auditorio*

Chairman: **Lars Larsen**

13.30-14.00 **Final conclusions and closure**

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Lectures

(L.1 - L.11)

L.1

ONE WORLD – ONE HEALTH. THE THREAT OF EMERGING PIG DISEASES: A EUROPEAN PERSPECTIVE

Thomas Blaha.

University of Veterinary Medicine Hannover Foundation, Germany.

Two major tsunami-like threats to human health shook veterinary medicine in the last two decades and resulted in two paradigm shifts: the BSE crisis and the occurrence of Avian Influenza that killed several people in Asia.

The occurrence of BSE in conjunction with the sudden increase of Salmonella Enteritidis in eggs and poultry products made clear that the traditional food safety assurance measures, veterinarians inspecting carcasses and food from obviously healthy animals before they are declared “fit for human consumption”, could not handle any more all food safety risks. Prions and pathogens as well residues that do neither cause clinical symptoms in the live food animals nor pathological gross lesions in the carcasses demanded for a new concept. The resulting food safety approach (Reg. [EC] 178/2002 and the “EU Hygiene Package”), focusing on the entire food chain with process optimization instead of end product inspection is best explained by the slogan: healthy food from healthy animals. This paradigm shift to the continuous improvement of all procedures along the entire production chain for food of animal origin from feed to food is accompanied by a deeper understanding of animal health, which nowadays includes also the freedom from or minimization of the occurrence of latent zoonotic pathogens and residues as well as the wellbeing of the animals, which can only be assured by applying best practice procedures in each phase of the production chain, with assuring a high animal health status and pre-harvest food safety being the major target in the animal production phase.

The occurrence of Avian Influenza with human casualties in Hongkong and later also in other regions of Asia with the threat to migrate to Europe alerted as much as the medical community world-wide, both human and veterinary medicine, as the occurrence of SARS. These events highlighted the somewhat forgotten or underestimated three facts about human health word-wide: a) one fourth of all deaths in humans are due to infectious diseases, b) two thirds of all infections in humans are zoonoses, and c) three fourths of all emerging diseases in humans are zoonoses. These epidemiological facts made crystal clear that infectious diseases do not respect political and geographical boundaries, seldom species boundaries and globalization has accelerated the speed of pathogen spread exponentially.

Following the paradigm One World – one Health, the new EU Community Animal Health Strategy 2007 – 2013 (“Prevention is better than Cure”) is asking for improved biosecurity, monitoring and surveillance, reduced and prudent use of antibiotics as well as for a closer cooperation between human and veterinary medicine.

L.2
ONE WORLD – ONE HEALTH. THE THREAT OF EMERGING PIG DISEASES: AN AMERICAN PERSPECTIVE

John Deen.

University of Minnesota, St Paul, MN, United States.

Introduction

Few concepts in the area of infectious disease control and management have gained as much currency as the idea of "One Health". This concept has been interpreted in a number of ways, but the most comment involves some level of expansionary involvement of disciplines, species, locations and societal sectors. It is driven by the recognition that diseases are crossing borders, crossing species and often crossing historic sanitary inhibitors (1).

Most of these discussions are driven by anthropocentric drivers that focus on the origin and control of emerging diseases that appear to have had a local transferral from another species to humans. Many of these are wildlife species that have either transmitted diseases directly to human populations or indirectly through intermediate domestic animal populations.

Though these concerns have been most often expressed about risks to human populations, the same risks and concerns exist for pig populations, not only in threats to the health of pigs, but broader threats to the economic health of pig farmers when those diseases are also potentially zoonotic. The concept that there is one worldwide pig population and that the threats of emerging diseases must be addressed by broad communities is irrefutable. However, it is often difficult to find a broad strategy that addresses this concern.

The difficulty of addressing such concerns is that reductionist principles are often contraindicated in control strategies. Synthetic approaches that cross disciplines, cultures and politics are needed, even though such approaches are outside the historic capabilities of researchers. We need to recognize that, at its root, the problem is one of managing the ecology of putative pathogens.

One can divide the type of emergent diseases into the following categories:

- Resident pig pathogens that change in pathogenicity or transmission likelihoods.
- Non-pig pathogens that enter pig populations
- Nonpathogenic zoonotic agents that enter pig populations

The drivers of emergence can be a function of two factors. The first is simply probalistic, in other words the threat was always present and the emergence is simply a function of time. The other, more commonly discussed driver of emergence is a change in the ecology of these infectious agents.

This latter argument, that the emergence of new infectious diseases is often a function of a change in the environment is difficult to test, but there is often evidence of changes in factors such as inter-species contact rates, host immunity factors and vector populations. However, it should also be realized that if the ecology of infectious agents has been a risk factor with the emergence of new diseases, the ability to prevent new diseases must focus on the same factors.

Not surprisingly, our pig industry is "ahead of the curve" in many aspects of the control of these emergent pig diseases. We need to recognize her strengths and build upon them. I would classify the challenges and opportunities into those three following major categories.

Biosecurity

Biosecurity has had a long history of emphasis within the swine industry and frankly, the emergence of new diseases is in areas where biosecurity is weak. Exposure to other species such as bats or rodents but also feral swine should be a major concern. This can be through direct contact, but also through consumption of feedstuffs containing animal protein, including bush meat. Other points of potential exposure include biologic compounds such as vaccines that may become infected with other pathogens. This species of most concern is humans, and methods of identifying at-risk individuals should be a future focus.

Biosecurity steps often fall down during times of social and economic distress. In many regions the movement of human populations due to war or persecution is aligned with livestock movement. These populations are often immunocompromised due to nutritional deficits as well as increases in a wide range of infectious diseases. In addition, basic sanitary methods and resources are not available. It should not be surprising that these can often be the sources of burgeoning pathogen populations that can spill over into wider communities.

In some ways it should be surprising that comingling of livestock species is gaining currency in parts of the agricultural community. Though efficiencies can be gained through the common use of space and feedstuffs across species such as ducks and pigs, the risks of cross species transferral of pathogens, and the inability, in many cases to maintain all in all out practices should cause some concern.

Biocontainment

In most cases the level of the biocontainment is closely related to the level of biosecurity, but it is an under-examined discipline. The prevention of the spread of an outbreak eons the borders of a farm or region should be examined in more detail. The swine industry has done a great deal in detecting and monitoring diseases in breeding stock, but emerging diseases, often without confirmatory tests has been a downfall. Symptomatic surveillance of animal populations and subsequent inhibition of movement of pigs and products should be re-examined.

Moreover, the biocontainment of agents within a population needs further focus. Biocontainment has historically had little of the economic drivers that biosecurity has had.

Though some barns now have filters for incoming air. Very few barns have filters for outgoing air. Likewise, the containment of pigs and semen, and, to a lesser extent, of pork and manure within the region may need further consideration.

It is likely that we are currently containing many small outbreaks of potential emerging pig diseases through the steps of biosecurity and biocontainment already in place in the pig industry. It is the failures that often attract our attention, but compared to many species, including humans, the successes are significant.

Biocontainment of emergent diseases between countries is a subject that also needs much further discussion. Biosecurity rules are often seen as unfair non-tariff barriers, but biocontainment rules are its inverse, and often difficult to impose due to adverse economic effects.

Population immunity

Though individual pigs can become infected with a new agent, it is the characteristics of the population that drive an epidemic that can be perpetuated within the immediate population and then to a wider population. Some of this is attributed to the characteristics of the innate immunity of individual piglets, taking into account the wide range of that immunity within a population.

However, the ability to transmit that disease between pigs should also be taken into account. Population density, concentration of immunocompromised populations, concurrent pathogen challenges, sanitation levels, aerosol concentration and agonistic behaviors have all been associated with transmission likelihoods.

It is likely that the introduction of novel pathogens into swine populations in various parts of the world is by no measure a rare event. Robust populations are less likely to allow these “sparks” to result in full-blown fires. Good stockmanship and healthy pigs may be one of the best defences that the swine industry actually needs.

Discussion

There is a basic argument that the trend line for emerging infectious diseases is going up (2). This is a difficult supposition to test, and yet, even if the risk is not going up, the threat is real and a major economic shadow always challenging the pig industry.

The pig industry has been successful, in many cases, due to its competitive model that has always driven it to new answers. These answers appear to be based not only on such competition, but also on cooperation across borders, disciplines and conceptual frameworks.

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2. Jones, K.E., et al. (2008). Global trends in emerging infectious diseases. Nature 451:990-994.

L.3

ONE WORLD – ONE HEALTH. THE THREAT OF EMERGING PIG DISEASES: AN ASIAN PERSPECTIVE

Suparlark Nantawan Na Ayudhaya, Roongroje Thanawongnuwech.

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Introduction

Several viral diseases including foot-and-mouth disease (FMD), classical swine fever (CSF) and Aujeszky's disease (AD) etc. are still endemic in Asia and sporadically re-emerge when facing with biosecurity and/or vaccination failures. Recently, FMD outbreaks in Japan and South Korea have caused major economic impacts in those countries as well as in their swine industry. CSF still causes high mortality in back-yards and small holders decreasing incomes and food security for many rural villagers and the virus may spill into the industrial farms causing more or less economic outbreaks depending on herd immune status. Similarly, AD seems to be under control but still endemic in most Asian countries. Those countries have been spending a lot of money on vaccination and test kits for prevention and control those infectious swine diseases.

Unavoidably, many emerging swine diseases including porcine reproductive and respiratory syndrome (PRRS) and porcine circovirus associated diseases (PCVAD) etc. have been introduced into Asian countries possibly from imported breeders and semen in the last 2 decades. Those diseases become endemic due to previously unrestricted animal movement and biosecurity failure between and within the countries. Recently, highly pathogenic PRRS (HP-PRRS) firstly recognized in China in 2006 has been spreading to Vietnam, Cambodia, Lao PDR and later in Thailand (1). Similarly, after the first appearance in China in 2004, a Chinese-like strain of porcine epidemic diarrhea (PED) is currently prevailing in Thailand since late 2007 (2) and in Vietnam since 2008. Factors involving in inter- and intra-country spreading will be discussed. However, animal movement among neighboring countries plays a major role in this incidence. These trans-boundary swine diseases provide Asian pig producers with a unique challenge in disease diagnosis and control. Swine veterinarians can better control those diseases by utilizing more suitable screening tests and acquiring a better knowledge of the pathogenesis and modern diagnosis. In addition, the changes in herd size and operational systems are further challenges in the disease control. This manuscript discusses on current emerging swine diseases in South East Asia including FMD, HP-PRRS and PED along with major factors involving in disease spreading and important means to prevent and control the diseases as 'One World, One Health' reflecting the impact of globalization on these trans-boundary swine diseases.

Foot and Mouth Disease (FMD)

The South East Asia Foot and Mouth Disease (SEAFMD) Campaign was launched in 1997 to coordinate a sub-regional control of FMD (3). Because of its serious direct effect on animal health and production and its indirect socio-economic effect on trade in animals and animal products, FMD is currently a major trans-boundary animal disease in the countries of mainland South East Asia. Control of FMD needs close collaboration among neighboring countries between the authorities and the involvement of relevant stakeholders such as farmers and livestock traders. The main strategy is to firstly implement a progressive FMD-free zoning approach or compartmentalization before extending to larger areas. However, selecting the FMD-free zoning or compartment can be problematic since the selected location may involve the way of living of the native residents on back-yard farming and should have natural barriers in order to prevent easy access of illegal animal movement into the area.

Spreading of FMD has long been recognized due to the role of animal movement both within disease affected countries and across the borders. Viral transmission occurs in a number of ways, mostly from animal to animal direct contact including semen and milk, aerosol "wind borne" spread, by non-affected host animals including humans, as well as by contaminated animal products. Contaminated objects and other mechanical vectors can also be responsible for disease transmission.

Vaccines are recognized as an important tool and play a major role in FMD control both endemic and non-endemic areas or when used strategically as an aid to outbreak control and eradication efforts. Limitation of FMD vaccines exists since vaccination with one FMD serotype does not confer cross protection against other serotypes. It should be noted that vaccination might help slowing an outbreak in piglets, but it seems highly advisable to implement additional measures like pre-emptive culling of in-contact pig herds since vaccination seemed to be effective in cattle and sheep, but was less effective in pigs. Moreover, vaccination approach as a control tool is not uniform in most countries since available FMD vaccines are routinely used in the vast majority of commercial herds at the producer motivation and expense. Back-yards and small holders become at high risk or hot spot of the virus source due to irregular or no vaccination practice. To increase overall national herd vaccination coverage, implementation of mandatory vaccination, vaccine subsidies, and mass vaccination initiatives should be implemented.

The trans-boundary nature of FMD suggests that significant FMD control depends on neighboring implementation of collaborative and robust multi-country disease control initiatives SEAFMD campaign based in Bangkok (3). Zoning system in chosen specific areas is recommended for FMD endemic country like Thailand to have a free FMD zone in the eastern part of Thailand. It is an early step creating FMD-free zone and will extend more to other selected areas in the near future. Communication and public awareness campaign are essential component for effective control of FMD. To ensure public support and political commitment from the government, traders and farmers support is also required since they are in contact with animals and should be equipped with correct knowledge and practices. Changing the behavior of key stakeholders and farmers to follow proper biosecurity measures is important to effectively limit disease transmission. More epidemiological investigations of outbreaks are needed to better understand the virus behavior in the field.

Highly pathogenic porcine reproductive and respiratory syndrome (HP-PRRS)

Porcine reproductive and respiratory syndrome virus (PRRSV) antigenic and genetic heterogeneities as well as quasispecies evolution are well described. In addition, co-existence of the mixed genotypes or more than one strain in the herd become potentially problematic to control since cross-protection among strains does not exist (4). Following the outbreaks of swine high fever (SHF) syndrome caused by highly pathogenic (HP)-PRRSV in China, many genetic variants of this virus have been characterized and recent data suggest that those variants were originated from the CH-1a strain isolated in the South of China (5). A novel nucleotide deletion in nsp2 found in those Chinese isolates initially linked to the virulence of the virus could possibly attribute to a combination of HP-PRRS and other pathogens such as classical swine fever virus (CSFV), porcine circovirus 2 (PCV-2) and probably other additional agents even a non-pathogenic pathogen like *Streptococcus suis* serotype 7 (6). The HP-PRRSV containing two discontinuous sequence deletions in the nsp2 gene, has initially occurred in 2007 and continued to cause problems in China, Vietnam (7) and the Philippines. Unavoidably, the HP-PRRSV eventually found in the Thai back-yard pigs closed to the Lao PDR border in August 2010 causing high mortality rate in all age groups. Based on epidemiologic evidence, the HP-PRRSV might gain its entry from the pig trade at the border areas since the spreading of the HP-PRRSV occurred in Northern and Southern Vietnam, in Lao PDR (June 2010) and then in Cambodia (July 2010) (Tung, personal communication). The trans-boundary spreading of the HP-PRRSV from Southern China to South East Asia demonstrates the biosecurity failure among neighboring countries particularly at the border areas. In addition, spreading of the virus within the country also confirms the failure of biosecurity control mostly on animal movement and contaminated vehicles.

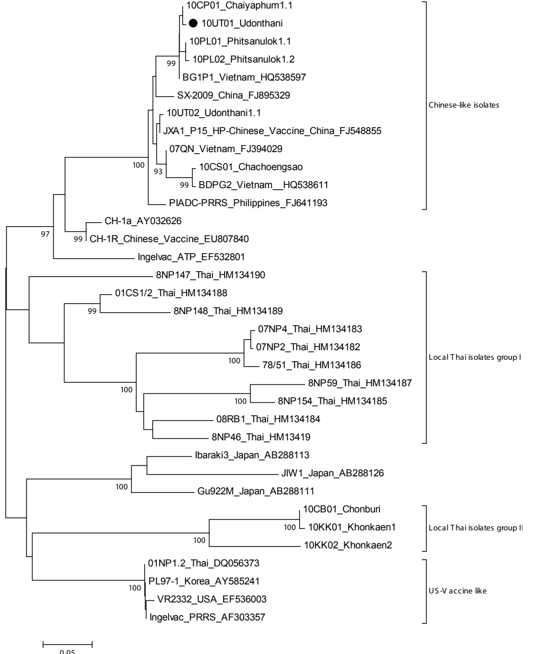

Figure 1. Phylogenetic analysis based on nucleotide sequence of NSP2 region. Dark dot represents the first Thai HP-PRRSV isolate in 2010.

Multiple introduction of HP-PRRSV into Thailand is evident based on the phylogenetic analysis (Fig. 1). It should be noted that the Ingelvac® PRRS ATP vaccine (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) is very close to the HP-PRRSV. A highly virulence PRRSV called atypical PRRSV emerged in the US in 1996 is the parent strain of that vaccine (8). Coincidently, the atypical PRRSV did cause 'sow abortion and mortality syndrome' similar to the Chinese HP-PRRSV. The evolution of this virus needs further investigation if the Chinese HP-PRRSV might mutate from the heavily used of the MLV vaccine.

Spreading of PRRSV among farms normally causes by introducing infected pigs or semen, while spreading within farms is mainly due to the intra-herd movement of the carriers or infected pigs or when mixing pigs during the production periods. Many intervention strategies to prevent its spreading both vertically and horizontally are the key components to control PRRSV. Continuous flow system commonly predisposes pigs to PRRSV infection after weaning creating the virus source circulating in the system. It should be noted that contaminated vehicles may play an important role on spreading of the virus within or among farms. Farmers should be educated on using disinfected, dry and clean vehicles and limiting of using vehicles among farms. Several management techniques implemented to control the spreading of PRRSV consist of reducing both vertical and horizontal transmissions including sow herd stabilization, all in/all out, medicated early weaning, segregated early weaning, and nursery depopulation as well as vaccination with limited success when applied to the HP-PRRSV. The effective means of disease prevention and control are rigorous bio-security. Eradication could be the ultimate tool for the HP-PRRSV control. Since current PRRS control strategies are not predictably successful, PRRS-associated losses will continue to be seen worldwide particularly when facing with the HP-PRRSV.

Porcine epidemic diarrhea (PED)

Porcine epidemic diarrhea (PED) was first identified in England in 1971 and has currently become a problematic disease causing massive economic losses in many countries, mainly, in Europe and Asia. It was not until late 2007 that re-emerged PED outbreaks appeared in central Thailand, an intensive pig raising area before spreading throughout the country (2). The losses caused by the recent PED outbreaks were very severe characterized by severe diarrhea and dehydration with milk curd vomitus in all naïve suckling piglets. Interestingly, pigs of all ages were affected showing varying degrees of diarrhea and off-feed depending on their ages. Interestingly, immunity from previous endemic PED virus strain and the immunity induced through the commercial Korean vaccines do not provide cross-protection (Personal observation). In addition, the immunity induced from gut-feed back to the sows and replacement gilts is not life-long. If persistence of the virus is diagnosed in consecutive litters of weaned piglets after an outbreak, virus elimination should be done by removing infected pigs immediately after weaning to another site for at least 4 weeks to avoid re-breaking with the virus. This emerging PEDV is currently prevailing and causing sporadic outbreaks in Thailand. Thus, it is recommended to encourage the PEDV specific immunity in all stocks when facing an acute outbreak and effective biosecurity is always the key management for PED prevention and control. Strict sanitary measures should be taken to prevent introduction of PEDV to the farm. Introduction of persistently infected pigs poses the highest risk, and disease can also be spread by human traffic.

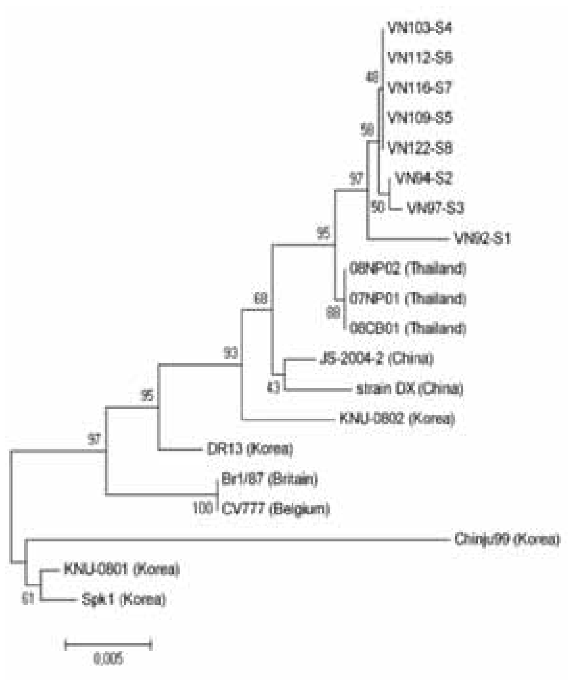

Figure 2. Phylogenetic analysis based on nucleotide sequence of the partial S gene of PEDV.

Similarly, emerging PED outbreaks confirmed by pathological features and RT-PCR occurred in the intensive pig raising area in southern provinces of Vietnam causing massive economic losses in 2009 (9). The severity of the clinical symptom appeared milder than the clinical signs found in affected piglets in Thailand. Phylogenetic analysis indicated that recent Thai and Vietnamese PEDV isolates originated from the same Chinese ancestor and they were gradually undergoing genetic variation and forming a new PEDV sub-cluster in each country (Fig. 2). Furthermore, these results suggested that the Chinese isolates (JS-2004-2) could be the ancestor of the current PEDV outbreaks transmitting to the other countries. It should be noted that the epidemiology of PED outbreaks in Thailand and Vietnam might not involve the animal movement at the borders since the incidents occurred in the center of the countries. In addition, Thailand does not import live pigs from China. Contaminated fomites and humans might involve in the route of transmission since sharing equipments, visitors and practitioners among regions are frequently seen in Asia. Until now, the route of PED introduction into Thailand or Vietnam has not been elucidated.

Spreading of PED outbreaks between farms within the country may cause by several major risk factors include poor biosecurity and improper application on fomites, animals and humans. Spreading among neighboring farms after the first PED introduction in the area is mostly due to animal and human movement as well as contaminated vehicles.

Conclusion

The impact of globalization on these trans-boundary swine diseases demonstrates that any emerging disease occurred in one country may emerge in another country sooner or later similar to the pandemic H1N1 2009 (10) depending on the pathogen nature. FMD virus transmission occurs in various means. Thus, FMD trans-boundary disease movement risk assessments should be considered and collaboration among neighboring countries, authorities, social network, stakeholders and farmers must identify risk factors in order to reduce FMD spreading. Fortunately, FMD vaccination may have a good impact on using to control the disease. Unlike FMD virus, the current HP-PRRSV control strategies are not predictably successful either using conventional control strategies or commercially available vaccines due to its severe immunosuppressive effects. Eradication and rigorous biosecurity could be the ultimate tool for the HP-PRRSV control. Similarly, the Chinese-like PED virus becomes endemic in Thailand and Vietnam and sporadically causes problems in the suckling piglets of subpopulation gilts and sows. PED vaccines are still not effective enough to provide fully lactogenic immunity to suckling piglets. Either eradication and rigorous biosecurity or maintaining the sow immunity by whole herd feed-back and later regular gilt acclimatization can be the alternative tool depending on the farm location. The World Organization for Animal Health (OIE) and Food and Agriculture Organization (FAO) should support on the design of surveillance and control methods for infectious trans-boundary animal diseases among countries.

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L.4

EPIDEMIOLOGY AND CONTROL OF PORCINE CIRCOVIRUS DISEASES WITH FOCUS ON POSTWEANING MULTISYSTEMIC WASTING SYNDROME

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Porcine Circovirus type 2 (PCV2)

Porcine Circovirus Diseases (PCVD) or Porcine Circovirus Associated Diseases (PCVAD) are terms referring to several disease entities, where Porcine Circovirus type 2 (PCV2) plays a significant role. Postweaning Multisystemic Wasting Syndrome (PMWS) is the most important PCVD. Other diseases where PCV2 infection is of importance together with other pathogens include reproductive disorders (64), Porcine respiratory disease complex (PRDC) (29), enteritis (30) and Porcine dermatitis and nephropathy syndrome (PDNS) (63). PMWS will be the focus of this paper.

PCV2 is regarded as a ubiquitous virus infecting most, if not all pig herds. Infection with PCV2 is necessary for PMWS to develop, but most research has shown that PCV2 needs one or more co-factors for PMWS to develop into severe and even fatal disease. Despite extensive laboratory investigations of PMWS affected pigs, no single viral cofactor has yet been identified (36), but several pathogens such as Porcine parvovirus (1), PRRSV (18) and *Mycoplasma hyopneumonia* (43) have been shown to enhance the severity of PCV2 infections.

PCV2 has been circulating in pigs for many years before being linked to disease (23).

Sequencing of the PCV2 genome has shown several different genotypes. Up till now 5 genotypes have been described and based on a proposal form an EU PCVD-consortium, they are named with letters starting with a, thus as of now a, b, c, d and e (53). Type a and b seem to have a world wide distribution whereas type c-e have only been found in Denmark (8), China (61) and Thailand (24), respectively.

Postweaning multisystemic wasting syndrome (PMWS)

Since its first description in high health herds in Canada in 1991 (17) PMWS has been reported from all major pig producing countries, except in Australia.

PMWS was first described in Europe (France) in 1996 (35), and during the following 5-10 years most European countries experienced an epidemic-like course of PMWS (58). During this epidemic most affected herds experienced heavy losses mainly due to a significant increase in post weaning mortality. Presently, the incidences of laboratory confirmed PMWS cases have decreased in most West-European countries (personal reports from UK, France, Spain and Denmark). This probably reflects a true decrease in PMWS incidence as reported from the field but may also reflect an increased confidence in clinical diagnosis among practitioners, reducing the number of requested laboratory diagnostic examinations.

During the present more endemic PMWS situation in Western-Europe, reports from the field indicate that the clinical manifestations has shifted from the classical PMWS symptoms with high mortality among weaners in the nursery to a more chronic form among finishers with more unspecific symptoms (unthrifty pigs, some increase in mortality, decreased productivity) and increased incidences of diseases caused by other pathogens. Interestingly, this trend to an older age of PMWS appearance has not been reflected in submissions to a diagnostic lab, at least not in Spain (54).

In North America the appearance of PMWS is most typically in the grower/finishers phase of the production, comparable to the more endemic situation in Europe now.

Diagnosis of PMWS

It is generally accepted that the PMWS diagnosis on herd level should be based on two conditions 1) a significant increase in mortality associated to clinical signs compatible with PMWS, and 2) an individual diagnosis in at least one out of 3-5 necropsied pigs (16). At necropsy, the typical macroscopic findings are wasting, non-collapsed lungs, pulmonary consolidation and enlargement of at least one lymph node. Microscopic findings in lymphatic tissue include lymphocytic depletion, histiocytic infiltration, inclusion bodies and giant cells (52, 56). Together with the microscopic lesions PCV2 antigen should be present in moderate to massive quantity in lymphoid tissues with typical lesions.

Data from a case-control study was used to validate the laboratory diagnostic set-up for PMWS (41). Based on necropsy of 3 unthrifty pigs from all herds in the case-control study, pigs with PMWS were found in 78% of case herds and in 26% control herd with no obvious clinical signs of PMWS. Thus, the clinical appearance (wasting & excess mortality) should be combined with the laboratory diagnosis to be able to classify a herd as PMWS affected.

Several studies have assessed the diagnostic value of using serology (antibodies) and PCV2 DNA detection (qPCR) for the diagnosis of PCVDs (14, 57, 66). Even though all studies found significantly higher viral load in PMWS pigs compared to non-PMWS pigs, they concurrently conclude that neither viral load nor antibodies can be used for diagnosing pigs or herds as PMWS affected because the diagnostic sensitivity and specificity are too low (14). Another reason for difficulties in setting up at common threshold for PCV2 DNA is the variance between different labs running qPCR (21).

Significance of PMWS

Post weaning mortality is one of the most significant losses in PMWS affected herds, but reduction in growth and poor feed utilisation as well as increased consumption of antibiotics add to the cost of the disease.

A study describing the first 43 cases of PMWS in Denmark showed an average post weaning mortality of 11% in the nursery (7-30 kg), ranging from a few per cent to more than 30% (19). Other studies comparing affected and non-affected herds showed an increase in mortality among weaners of 8% and 4%, and among finishers of 2% and 3.7% in Denmark and Spain, respectively (37, 41). These mortality rates reflect the mortality at a given time, but do not give information of the total loss during an outbreak of PMWS. However, a Danish study performed in 50 PMWS affected herds with a laboratory verified diagnosis, showed that the weaner mortality increased above the average national level (3.8%) already 300 days before the time of diagnosis, peaked at the time of the diagnosis (10.3 %) and stayed above the national level until 300 days after the time of diagnosis (2).

In a case-control study PMWS herds experienced a lower weight gain of 36 g/day in weaners and 52 g/day in finishers, compared to non-affected herds (41).

The impact of PMWS on feed utilisation has been illustrated in a USA vaccine trial (22). In the vaccinated groups of pigs the daily gain-to-feed ratio increased significantly by 1.5% (396 g/kg feed in vaccinates versus 360 g/kg feed in controls).

Two Danish studies have illustrated the impact of PMWS on the usage of antibiotics (26, 60). Both studies showed a significant increase in the consumption of antibiotics before and up till one year after the time for the diagnosis by the use of register data from a national database (VETSTAT) with information on all antibiotics used on all pig farms each month.

Transmission of PCV2 and PMWS

Several studies have focussed on the infection dynamics and transmissibility of PCV2 and of PMWS.

In a longitudinal study performed in PMWS affected herds, following cohorts of piglets at certain time points from birth to development of PMWS, showed that the PCV2 viral load in sera, nasal and rectal swabs and in lymphoid tissues were positive and significant correlated (14). These findings are in accordance with a study showing that PCV2 is shed in similar amounts by nasal, oral and faecal routes at least until 209 days post farrowing (47).

Experimental studies on boars have shown excretion of PCV2 virus in semen continuously until at least 50 days after inoculation of the boars (40). No differences in the shedding patterns were observed between PCV2a and PCV2b strains. A study in boar studs in USA found positive pooled serum samples (qPCR) in 12 out of 17 boar studs (27).

Sampling of colostrum and serum from 125 sows and pre-suckle piglet serum (3-5 pigs per sow) in 5 commercial breeding herds with no PCVD reported, showed high levels of PCV2 virus DNA in colostrum as well as in sow and piglet serum (40-47 % positive samples) (55). PCV2b was detected at a considerable higher frequency than PCV2a and concurrent PCV2a/PCV2b infections were detected to some extend (6-12% of samples).

Thus both vertical and horizontal transmission of PCV2 seems likely to occur.

Investigation of the spatial (location of herds) and temporal (time of diagnose) pattern of Danish pig herds diagnosed with PMWS during the first two years after the first herd was diagnosed identified one spatio-temporal cluster between February and May 2002 (58). The identification of a significant spatio-temporal cluster early in the epidemic supports the hypothesis that PMWS is caused by a "new" pathogen initially introduced to one or a few naïve pig herds and subsequently spread to most parts of Denmark during the first two years after the introduction. These findings are supported by study of Dupont et al, 2008 (8) showing a contemporary shift in genotypes from PCV2a to PCV2b in Denmark. This shift in the prevalent genotypes also happened in USA and Canada (12). One case report from two Spanish farms supports, on farm level, this link between simultaneously appearance of PMWS and a genotype shift from a to b (7). Furthermore, some experimental data indicates that different isolates of PCV2 may differ in virulence (45) but no genuine virulence marker of PCV2 has yet been identified.

Transmission of PMWS from diseased pigs to healthy pigs after mingling, have been studied in two transmission experiments (33). Both studies using PMWS affected and non-affected pigs from commercial herds, concluded that PMWS can be transmitted to healthy pigs after mingling with pigs from PMWS affected herds, especially at relatively close contact (pen mates or between pigs in neighbour pens). These results are supported by a similar study in New Zeeland (25). Airborne transmission of PMWS has been shown in an experimental study (32). The conclusions from these transmission studies points at the importance of optimal internal as well as external bio-security to reduce the prevalence of PMWS.

Risk factors for PMWS

Epidemiological studies comparing affected herds with non-affected herds have been carried out in UK, France, The Netherlands, Spain and Denmark with the objective to identify factors that either increased or decreased the risk for a herd to be affected by PMWS (6, 9, 37, 49, 50, 59, 63, 65). The most significant factors identified in these studies were:

Factors that INCREASES the risk for a herd to be affected with PMWS	Factors that DECREASES the risk for a herd to be affected with PMWS
PRRS: - Infection or vaccination - In Denmark only the US-strain of PRRS	High level of external biosecurity: - Quarantine for purchased pigs and gilts - Change of boots/clothes in entrance room of the farm - Delivery of finishers through delivery room
Other affected herds in the area	Long empty period (weaners and sows)
Purchasing larger amounts of replacement gilts (> 500 per year)	Dry sows in collective pens
Herd size > 400 sows	Treatment of external parasites
High seroprevalence of PCV2 antibodies	Vaccination of sows against atrophic rhinitis
PPV antibodies among finishers	
Active PPV infection in pregnant dams	
On farm semen collection and AI	
Visitors without a 3 day pig-free period	

Several studies have focussed on risk factors for PMWS at the individual pig level. Pigs with low PCV2 antibody titers at 7 weeks of age (and no subsequent seroconversion) and piglets born by seronegative sows were at higher risk of being affected by PMWS (HR=7.0 and 2.8, resp.) (50). Likewise active infection of the pregnant sows with parvovirus increased the risk (HR=2.3). Calsamiglia et al, 2007 (4) found that more piglets died from viremic sows than from non-viremic sows (OR=2.1) and from sows with low antibody titers (OR=3.0). A longitudinal study in 7 PMWS farms showed increased risk of PMWS if piglets were infected early (before 7 week of age), whereas reduced risk was found if piglets were weaned after 21 days and if they were born of seropositive sows (51). The significance of maternal immunity as protective for disease development, as indicated in these studies, is supported by a longitudinal cohort study in 13 Spanish/Danish PMWS farms (15).

Control of PMWS

Concerning control of PMWS there is a time before and a time after the emerging of commercial PCV2 vaccines in the period 2004-2006. Before the vaccines became available, much focus was on good production practice and on the control of other diseases (38). The timing of other vaccines seemed to play some role in preventing or reducing the problems with PMWS (42, 44). Different reports from the field and one clinical trial indicated that serum from pigs recovered from PMWS by injection could prevent PMWS to some extend (20).

Introduction of the PCV2 vaccines indeed changed the world and a huge amount of conference abstracts and peer-reviewed articles have shown great benefit of the vaccines. The success of the vaccines is probably based on activation of both the humeral and cellular immune responses against PCV2 (28). Presently 4 commercial PVC2 vaccines are commercial available in most countries; one sow vaccine (Circovac[R], Merial) and 3 piglet vaccines (Ingelvac[R] CircoFlex, Boeringer Ingelheim; Porcillis[R] PCV/Circumvent[R] PCV, Intervet/Merial & Circovac[R], Merial). All vaccines are inactivated and based on a genotype PCV2a strain. One vaccine has been withdrawn from the market (Suvaxyn[R] PCV2, Fort Dodge/Pfizer).

One extensive meta-analysis on the effect of vaccines against PCV2 identified 107 studies from 2007-2008 of which 24 studies of relevant quality were included in the analysis (34). A significant effect of vaccination was seen on average daily gain (ADG). The increase in ADG was 41.5g for finishers, 33.6g for nursery-finishers and 10.6g for nursery pigs. Likewise a significant effect of vaccination was seen on reduction in mortality. The reduction for finishers were 4.4% and for nursery-finishers 5.4%. No differences were found in effect on ADG and mortality of the different vaccines.

Even in farms with a subclinical level of PCVD and with acceptable mortality rates, vaccination of piglets has shown to increase ADG by 32g among grower-finishers (from day 41-131) (31).

One vaccine trial (Circumvent[R] PCV) showed a significant increase in daily gain-to-feed ratio by 1.5% (6g) in vaccinated pigs (22).

The timing of the vaccination of the piglets is often in question due to the possible interaction of maternal derived antibodies (MDA) that in many studies have been shown to protect against development of PMWS (see earlier). In an experimental setup where 3 week old pigs were vaccinated (Porcillis[R] PCV) and challenged with a PCV2b three weeks after vaccination, high level of MDA at the time of vaccination were found to interfere with the active seroconversion of the piglets, even though the vaccine significantly reduced viremia and shedding of virus (11). One study using Ingelvac[R] CircoFlex showed no difference in efficacy whether pigs were vaccinated at 3 week or 6 weeks of age indicating no significant impact of MDA (5). Never the less, even though some discrepancies exist on the inhibiting effects of MDA, it is recommended to avoid vaccination of too young piglets.

Concerning the question on using sow and/or piglet vaccination, one clinical field trial in one herd showed similar good efficiency among three different vaccination protocols (Circovac[R]) – vaccination of sows, vaccination of piglets and vaccination of both (48). An experimental study indicated that sow vaccination and piglet vaccination had similar reducing effect on the viral load in piglets (46). Sow vaccination reduced the prevalence of sows with PCV2 in colostrum compared to non-vaccinated sows (13). One study showed an increase in weaning weight by 0.93 kg in piglets born from vaccinated sows (3).

It has been speculated if the efficacy of the commercial vaccines based on PCV2a might be jeopardised by the fact that most infections are caused by PCV2b strains. Based on an experimental study this seems not to be the case (10). Based

on market research the piglet vaccination rates on the global marked has been estimated (39). In Europe examples on countries with a high vaccination rate (> 80%) is Germany, UK, Ireland, Austria, and Switzerland whereas Russia, Denmark, and Poland have a low rate (< 30%). USA, Canada, Mexico Brazil and Chile have a very high rate (80-98%). In Asia Korea and Japan has high rates (70-90%) whereas China and Vietnam have low rates (<5%). Interestingly, 34% of the piglets seem to be vaccinated in Australia, where PMWS has not yet been diagnosed.

Whether this extensive use of PCV2 vaccines has changed the epidemiology of PCVDs apart from reducing the losses related to classical PMWS, is still an open question since the data on the prevalence and impact of the various PCVDs are scarce. Thus, more research on the role of PCV2 on enteric, reproductive and respiratory diseases in general should be performed.

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L.5

EMERGING AND RE-EMERGING VIRUSES IN SWINE

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Introduction

In the past two decades or so, a number of viruses have emerged in the global swine population. Some, such as porcine circovirus type 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV), cause economically-important diseases in pigs, while others such as Torque teno sus virus (TTSuV), porcine bocavirus (PBoV), porcine toroviruses (PToV), and porcine lymphotropic herpesviruses (PLHV) are mostly subclinical in nature in swine herds. Although some emerging and re-emerging swine viruses such as swine hepatitis E virus (swine HEV) and porcine sapovirus (porcine SaV) may have an unknown clinical implication in swine health, they do pose a human public health concern due to confirmed (swine HEV) or potential (porcine SaV) zoonotic risks. This talk will focus on the emerging swine viruses that currently have uncertain clinical and economic impact on pig health. Those emerging viruses causing known major swine diseases such as PRRSV and PCV2 will not be discussed in this talk.

Swine hepatitis E virus (swine HEV)

Since the discovery of swine HEV in 1997 from pigs in the United States (1), the virus has now been identified in swine herds from essentially all swine-producing countries of the world (2). Swine HEV is currently classified in the family of *Hepeviridae*, which consists of at least 4 genotypes of human HEV, avian HEV and other animal strains of HEV. Thus far, all swine HEV strains identified from pigs worldwide belong to either genotype 3 or 4, and are genetically closely-related to, or in some cases indistinguishable from, genotypes 3 and 4 strains of human HEV (3).

Swine HEV infection is widespread in swine farms worldwide from both developing and industrialized countries regardless whether HEV is endemic in respective human populations (2). The virus generally infects pigs of 2-4 months of age with a transient viremia lasting 1-2 weeks and fecal virus shedding for about 3-7 weeks. Swine HEV infection in pigs is subclinical, although microscopic lesions of hepatitis have been found in both naturally- and experimentally-infected pigs. Mild to moderate multifocal and periportal lymphoplasmacytic hepatitis were observed in naturally-infected pigs. Pigs experimentally-infected with swine HEV had mildly-to-moderately enlarged hepatic and mesenteric lymph nodes, and multifocal lymphoplasmacytic hepatitis and focal hepatocellular necrosis (4).

The major concern currently for swine HEV is zoonotic human infection, pork and environmental safety (5). Hepatitis E is a recognized zoonotic disease, and pigs are reservoirs for genotypes 3 and 4 HEV. Under experimental conditions, genotypes 3 and 4 strains of human HEV infected pigs, and conversely genotypes 3 and 4 strains of swine HEV infected non-human primates. Swine veterinarians and other pig handlers are at increased risks of HEV infection, and individuals from traditionally major swine-producing states in the USA are more likely to be positive for HEV antibodies than those from traditionally non-swine States. Approximately 11% of the pig livers sold in grocery stores in USA and 2% in Japan are positive for HEV RNA, and most importantly, the contaminating virus in the commercial pig livers remains infectious. The virus sequences recovered from commercial pig livers are closely related, or identical in a few cases, to the viruses recovered from human hepatitis E patients.

Sporadic cases of acute hepatitis E have been definitely linked to the consumption of contaminated raw and undercooked pork. In France, figatelli pig liver sausages have been identified as the source of sporadic cases of hepatitis E in humans (6). In Japan, cluster cases of acute hepatitis E have been reported in patients who ate swine HEV-infected wild boar meats. As a fecal-orally transmitted disease, contaminated water is the main source of HEV infection. Pigs infected by swine HEV excreted large amounts of viruses in faeces, which poses a concern for environmental safety. Infectious swine HEV has been detected in swine manure, and in concrete pits and lagoons of swine manure storage facility. Thus, swine manure land application and runoffs could be the source for contamination of irrigation and drinking water or costal water with concomitant contamination of produce or shellfish. Swine HEV was detected in oysters, and consumption of contaminated shellfish has been implicated in sporadic cases of acute hepatitis E (7).

Although swine HEV does not pose a major health concern in pigs, it remains to be determined if concurrent infections of swine HEV with other swine pathogens could have any synergistic effects on pig health. The demonstrated zoonotic, pork and environmental safety risks associated with swine HEV infection in pigs indicate that it is important to eliminate swine HEV from commercial productions.

Torque teno sus virus (TTSuV)

Porcine Torque teno virus (TTV), now known as Torque teno sus virus (TTSuV), was first identified in Japan in 2002 from domestic pigs (8), even though evidence of TTSuV infection was traced back to as early as 1985 in Spain (9). TTSuV is a small single-stranded circular DNA virus in the genus *Iotatorquevirus* of the family *Anelloviridae*, which also comprises its homologous counterpart of human TTV. At least two species of TTSuV, TTSuV1 and TTSuV2, have been identified from pigs worldwide.

TTSuV appears to be ubiquitous in both healthy and diseased domestic pigs worldwide (10). Co-infections with TTSuV1 and TTSuV2 at high prevalence rate have been documented in pigs worldwide by using PCR and real-time PCR assays (11-13). By using the putative capsid protein as

the ELISA antigen, a high rate of seropositivity to TTSuV2 was detected in conventional pigs of various sources but not in gnotobiotic pigs (14). In general, pigs with undetectable TTSuV2 DNA were more likely to have a lower anti-TTSuV2 antibody level (14). Multiple infections of TTSuV with distinct genotypes or subtypes of the same species in the same pig have also been reported (15).

The pathogenicity of TTSuV in pigs remains debatable. In a gnotobiotic pig model, TTSuV1-containing homogenates partially contribute to the experimental induction of porcine dermatitis and nephropathy syndrome (PDNS) and postweaning multisystemic wasting syndrome (PMWS) (16-17). In addition, it has been shown that PMWS-affected pigs with low or no detectable PCV2 infection had a higher prevalence of TTSuV2 DNA than non-PMWS-affected pigs in Spain (18), although no significant differences in viral loads of both TTSuV1 and TTSuV2 were found in a small sample size study in Korea between PCV2-negative pigs and PMWS-affected pigs. Interestingly, PMWS-affected pigs had a significantly lower level of TTSuV2 antibody than PMWS-unaffected pigs. Vertical transmission of TTSuV has been reported, however there were no statistically significant differences in TTSuV prevalence between aborted fetuses and fetuses collected at slaughterhouse (19).

The lack of a susceptible cell culture system to propagate TTSuV and the difficulty in obtaining TTSuV-negative conventional pigs for research greatly hinder our ability to understand the pathogenicity of TTSuV in pigs. It remains to be determined if TTSuV has any adverse effect on pigs concurrently infected with other swine pathogens.

Porcine bocavirus (PBoV)

PBoV was discovered in 2008 from pigs in Hong Kong (20), and is genetically related to human parvovirus 4 with approximately 60% nucleotide sequence identity. Approximately 44% of the lymph nodes, liver, serum, nasopharyngeal and faecal samples from pigs in Hong Kong are positive for PHoV DNA. The virus appears to be widespread in swine herds worldwide, and PBoV has been identified form pigs in various countries including Sweden, the United States, and China (21). The pathogenicity of PBoV in pigs is unclear. In Sweden, in addition to TTSuV and PCV2, approximately 88% of the PMWS-affected pigs were positive for PBoV DNA, although 46% of the pigs without PMWS are also positive (22). It remains to be determined if PBoV plays any role in pathogenicity during concurrent infections with other swine pathogens.

Porcine lymphotropic herpesviruses (PLHV)

In 1999, by using a pan-herpesvirus consensus PCR assay, two closely related gamma-herpesviruses, designated porcine lymphotropic herpesviruses 1 and 2 (PLHV-1, and PLHV-2), were discovered in pigs (23). A third porcine gamma-herpesvirus with considerable sequence differences with PLHV-1 and PLHV-2, designated PLHV-3, was identified in 2003 (24). All three viruses were frequently detected in the blood and lymphoid organs of domestic pigs from different geographic regions. Propagation and isolation of PLHV in cell culture are not available. Molecular epidemiological data suggested that PLHV infection is ubiquitous in commercial swine herds, and PLHV DNA was frequently detectable in the blood, spleen, and lung tissues. In addition to domestic pigs, PLHV DNA was also detected in high frequency from miniature and feral swine (25).

The pathogenicity of PLHV in pigs under natural conditions remains unclear. It has been reported that PLHV-1 is associated with post-transplant lymphoproliferative disease (PTLD) in miniature pigs following allogeneic hematopoietic stem cell transplantation (26-27). The clinical symptoms of experimental porcine PTLD, such as fever, lethargy, anorexia, high WBC count and palpable lymph nodes, are similar to those of human PTLD, which was linked to a human gamma-herpesvirus, Epstein-Barr virus. Characteristic gross pathological lesions in PTLD pigs include enlargement of tonsils and lymph nodes. Microscopic lesions include typical polymorphous PTLD cells with a mixture of immunoblasts, plasmacytoid cells, and plasma cells in the lymph nodes.

Currently, the main concern for PLHV is the potential risk of human infection in xenotransplantation with pig cells, tissues and organs. Appropriate breeding procedures can eliminate PLHV, and piglets free of PLHV were produced via caesarian-derived and barrier-reared breeding procedure (28).

Porcine torovirus (PToV)

PToV was identified in 1998 from piglets in the Netherlands (29), and belongs to the genus *Torovirus* of the family *Coronaviridae* in the order *Nidovirales*. PoTV is genetically related to bovine and equine toroviruses with 60-70% sequence identities. PToV has been identified from piglets in many countries including The Netherlands, Belgium, Hungary, Korea, Spain and Italy (30). Seroprevalence of PToV varied from 50-80% depending on the ages and geographic origins of piglets (29, 31). Piglets shed virus in the faeces for 1 or more days, starting 4 to 14 days after weaning (29), and about 19-40% of the piglets had fecal virus shedding (32-33). The lack of an *in vitro* cell culture to propagate PToV hinders our ability to determine the pathogenicity of PToV in pigs, and therefore the pathogenic potential of PToV as diarrhea-causing agent in pigs remains unclear.

Porcine sapovirus (porcine SaV)

Porcine SaV was discovered in 1980 by EM as rotavirus-like and calicivirus-like virus particles associated with piglet diarrhea (34), and genetic characterization of the virus in 1999 led to its classification as a sapovirus (35). Porcine Sav belongs to the genus *Sapovirus* of the *Caliciviridae* family. At least 5 distinct genogroups of sapoviruses have been identified: human SaV belongs to GI, GII, GIV, and GV, whereas porcine SaV primarily belongs to GIII although potential new genogroup of porcine SaVs and recombinant SaVs have also been reported in pigs and humans (36-37).

Porcine Sav appears to be widespread in the pig population worldwide, and has been genetically identified from feces of pigs in numerous countries including United States, Denmark, Finland, Hungary, Venezuela, Italy, Spain, Japan, Slovenia, Canada, Brazil, Korea (38). The prevalence of porcine SaV varied from herds to herds and among different geographic locations with the highest prevalence rate of 62% in the United States (40) and the lowest prevalence rate of 2% in Hungary (38). The highest prevalence was seen in young piglets of 2-8 weeks of ages, and the GIII genogroup is most prevalent (38).

Sapoviruses are associated with diarrhea in humans and animals (pigs and minks) (36). The Cowden strain of porcine SaV was shown to induce diarrhea and intestinal lesions in experimentally-infected gnotobiotic piglets (40). However, in a recent study, no significant difference was observed in the prevalence of porcine SaV between healthy pigs and pigs with diarrhea in Spain and Denmark (38).

Definitive evidence of zoonotic human infection by porcine SaVs is still lacking. However, the demonstrated intra-genogroup and inter-genogroup recombination events between sapoviruses raise a potential concern for cross-species infection (41).

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L.6
SWINE INFECTING TORQUE TENO SUS VIRUSES

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Introduction

Torque teno viruses (TTVs) are small, non-enveloped viruses with a circular single-stranded DNA genome, belonging to the family *Anelloviridae*. These viruses are known to infect several vertebrates including human, non-human primates, dogs, cats, wild species and farm animals as pigs. Most of the current knowledge of Anelloviruses is based on research done with human TTVs, but during the recent years research on swine viruses has been initiated in several laboratories wordlwide.

Viral properties

Anellovirus virions are icosahedral, non-enveloped, with a diameter of 30-32 nm. In swine, two species have been identified so far, *Torque teno sus virus 1* (TTSuV1) and *2* (TTSuV2) belonging to the *Iotatorquevirus* genus. The genome of these viruses is small, about 2.8 Kb. TTSuV species are genetically very diverse differing from each other more than 50%. Intraspecies nucleotide variation is up to 30% within TTSuV1 and somewhat lower within TTSuV2 (15%) (1,2). Despite their high genetic divergence, the genomic organization is similar between both viruses, with a coding region containing three overlapping open reading frames and an untranslated region containing the viral promoter and regulatory sequences necessary for transcription and genome replication. (1,2) Protein sequences contain short regions highly conserved in the replication proteins of circular viruses.

Epidemiology

TTSuVs are transmitted both horizontally and vertically. Nasal and faecal samples can be DNA positive already in one week-old piglets (3). While prevalence in these samples increases with the age of animals, faecal excretions of 15 week-old pigs is rather low (15%) and nasal detection at the same age animals is higher (30% for TTSuV1 and 55% for TTSuV2). Vertical transmission is also occurring since the viruses have been detected in foetal tissues and blood, semen and colostrums (4,5,6,7). Due to efficient transmission routes, TTSuVs are ubiquitous and distributed worldwide. However, a recent study indicates that certain subtypes are dominant in different geographic regions (8).

Tissues from pigs up to 5 weeks of age and foetuses can be negative or contain low amounts of virus. Tissues from older animals have been found to contain high amounts of both TTSuVs and highest viral prevalence (3,7). Therefore, it seems that infection with TTSuVs leads to a progressive persistent infection that begins at early stages of life (foetus), with increasing prevalence and viral load in tissues with age. High viral loads in the oldest analyzed animals might be indicative of an inefficient immune response against the virus.

Currently, no cell culture system for TTSuV propagation exists. The diagnostic of these infections is mainly based on end-point PCR but lately a semi-quantitative PCR for viral DNA load definition in tissue samples (7) and several quantitative PCR methods from serum have been reported (9,10,11).

Disease association

TTV infects a relatively high proportion of animals that are apparently healthy (10). Therefore, it seems that TTV infection by itself does not cause immediate disease. However, it is believed that TTSuVs can influence the development of some diseases or even affect their outcome.

Evidence for TTSuV disease association has been lately accumulated, especially in regards to porcine circovirus diseases (PCVDs) (12). Specifically, it has been shown that TTSuV2 prevalence is higher in postweaning multisystemic wasting syndrome (PMWS) affected pigs than healthy animals. Such difference was not evident with TTSuV1 (13) Additionally, utilizing quantitative PCR technique it has been shown that TTSuV2 viral loads were significantly higher in PMWS affected animals than in healthy animals while TTSuV1 loads were not related with the PCVDs (11). On the contrary, one study concluded that both species did not seem to be potential agents able to aggravate PMWS. However, this study used only 11 PMWS affected and 11 healthy pigs (10).

On the other hand, gnotobiotic pigs inoculated with a tissue homogenate containing TTSuV1 7 days prior to PCV2 challenge developed PMWS under experimental conditions (14). In addition, a PDNS-like condition has also been reproduced by means of the concomitant inoculation of PRRSV and a PRRSV-negative tissue homogenate containing TTSuV1 (15). Therefore, it seems that TTSuV species are not only genetically distinct but also pathologically.

The likelihood of TTSuV co-infection with PCV2 under field conditions is extremely high, while in most of the cases such co-infection does not lead to a disease but is subclinical. Therefore, the disease association is possibly not only a matter of co-infection, but of viral load. It may be that some TTSuVs are benefiting the disease status of its host by increased viral release or replication. TTSuV viremia may be associated with the level of immunocompetence of the animals and therefore it could be uncontrolled in PMWS animals, which are known to be immunocompromised.

Conclusions

Despite of the recent interest and increasing amount of publications, there are still several issues on TTSuVs to be investigated. One of the most interesting subjects is the disease association; as already suggested by recent publications it is likely that TTSuVs differ in their virulence depending on the viral

species/types. One species/type might be more disease-linked than others and co-infection with other viruses could affect the outcome or progression of some diseases, as already shown in human TTVs. The limitation in the current research is the lack of various techniques like *in-situ* hybridization and immunological tools. Due to the high genetic variability, the viral species/types under investigation should be always characterized allowing their possible associations to biological properties.

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L.7

BETTER MOLECULAR INSIGHTS IN THE PATHOGENESIS OF PRRSV INFECTIONS AND IMMUNE RESPONSE SHOW THE WAY FOR MORE EFFICIENT PRRSV VACCINES

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Porcine reproductive and respiratory syndrome virus (PRRSV) is a major porcine pathogen, causing huge losses in swine industry since the end of the eighties. American and European type attenuated vaccines were quickly introduced in the nineties, with good results. Inactivated vaccines were also registered but were only able to boost an existing immunity. In the beginning of the nineties, the isolates in Europe and North America were genetically homogeneous. However, over the next decade the viruses started to diverge. This led to the appearance of PRRSV strains that were more difficult to control by existing vaccines and that were more virulent. At present, it is extremely difficult to develop safe and efficacious vaccines with a broad activity. The traditional approaches of trial and error/success are no longer very successful. That is where the quest for new generation PRRSV vaccines starts. A veterinary fundamental research-oriented approach is the only one that can result in better vaccines for controlling PRRS in a long term. Better insights in the pathogenesis and the immune response will show the way.

Hallmarks of the pathogenesis of PRRSV infections are the specific tropism of the virus for differentiated macrophages (lungs, lymphoid tissues and placenta) and persistence due to immune evasive and immune modulating properties of the virus. The specific tropism can be explained by the beautiful interplay between viral and macrophage molecules. It all starts with an interaction of the GP5/M complex in the viral envelope with heparan sulphate moieties on cellular glycoproteins anchored in the plasma membrane. These weak virus-sugar interactions are allowing the virus to glide through the roof of foliage of the “sugar forest” till it finds its real receptor, sialoadhesin. Sialic acids on the GP5/M complex are binding to this lectin. Binding of the virus to sialoadhesin activates a clathrin-mediated internalization. The viruses end up in early endosomes where a pH drop is necessary for the disassembly together with CD163 and proteases (cathepsin E and/or a serine protease). After release of the nucleocapsid in the cytoplasm, translation and transcription, assembly and egress follow.

The immune response is completely not efficient in controlling virus replication. The virus developed in evolution a certain number of anti-immune tricks to escape from elimination. This is already clear at very early stages of infection. Extremely low levels of interferon alpha are produced. The humoral immunity starts like with other viruses. Antibodies against the nucleocapsid can be found at one week of infection. In this perspective, nothing goes wrong. High titers are reached and a normal isotype switch is detected. However, antibodies against envelope proteins, under which neutralizing antibodies, are raised at later stages. Glycosylation and decoy activities are forwarded as main reasons for the late appearance of neutralizing antibodies. Once they appear, neutralizing antibodies are very helpful in eliminating cell-free virus. However, the antibodies cannot eliminate infected cells because viral envelope glycoproteins are not expressed on the plasma membrane. They are retained in the endoplasmic reticulum and as a consequence the antibody-dependent cell lysis by complement and phagocytes is not occurring. By pepscan analysis of the envelope glycoproteins of European PRRSV strains, it was demonstrated that porcine anti-PRRSV antisera recognize 21 antigenic regions and that pig differences exist in the recognition pattern. After purifying peptide-specific porcine antibodies, functional analyses were performed. Porcine antibodies that recognize the peptide aa 57-68 of GP4 are strongly neutralizing PRRSV, which was interesting in the direction of vaccine development. However, due to a huge variation in this region cross-neutralization did not occur, burying the vaccine dreams. In addition, two neutralizing antigenic regions were found in GP2, two others in GP3. One neutralizing antigenic region in GP3 is very conserved and neutralizing antibodies against this region were found in most infected pigs. This is an interesting region for vaccine development. No neutralizing antigenic regions were found in M and GP5. The cell-mediated immunity is delayed in comparison with other viral infections. Cytotoxic T-lymphocytes are not eliminating PRRSV-infected macrophages and NK cells are suppressed in their cytotoxic function. However, a not yet defined subpopulation of leukocytes is able to efficiently lyse PRRSV-infected macrophages.

One has to take care with different factors in the development of vaccines and vaccination programs for controlling PRRS. First of all, due to genetic changes, it is essential to have adaptable vaccines. Secondly, more information is needed on cross-protection between strains. This will allow to determine how many and which strains should be enclosed in the vaccine that will be used in certain regions/countries. Very important are the links between genotype-serotype-protectotype. The “one-vaccine-will-do-it-all” vision of some people should be abandoned for PRRSV. Thirdly, the immunological arms that are still working in controlling virus replication in pigs should be identified and should be activated by vaccines. For instance, neutralizing antibodies are involved in protection. Therefore, they are important to be induced. Concerning cell-mediated immunity, more research is needed to identify the active subpopulation of leukocytes and how they can be stimulated. Fourthly, it is important to define at a herd level what is needed to control circulation. For instance, vaccinating naïve animals (negative gilts, piglets after losing their maternal immunity) should preferably be done with attenuated vaccines or vector vaccines. To this purpose, low vaccine virus titers are sufficient. Boosting the immunity, induced by attenuated vaccines or infection, should preferably be done by a vaccine with a high antigenic mass. Attenuated vaccines with a high virus titer and inactivated vaccines with a high dose of correctly inactivated PRRSV are suitable. Important tools to set up successful vaccination programs are (i) adaptable inactivated vaccines, (ii) adaptable attenuated vaccines or vector vaccines, (iii) cell lines to grow PRRSV to high titers and (iv) ELISAs to discriminate infected animals in a vaccinated herd.

The numerous breakthroughs that recently were made in the pathogenesis of PRRSV infections and the immune response/ modulation are the light at the end of the tunnel for vaccine developers.

L.8

THE PRRS COORDINATED AGRICULTURAL PROJECT (CAP): THE CONTROL AND ELIMINATION OF PRRS

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In 2003, the United States Department of Agriculture (USDA) established the PRRS CAP (www.prrs.org), a program project that integrates the collective talents of scientists, veterinarians, producers, and allied industry partners to develop innovative strategies for the control and eventual elimination of PRRS. The effective control and elimination of PRRSV is dependent on understanding its complex biology/ecology combined with the development of tools for detection and control, which can be applied to the field. The current four year program (2008-2012) is supported by a US $4.9 million grant from USDA. PRRS CAP goals and activities are the result of extensive stakeholder input from scientists, represented by universities and government laboratories; veterinarians, including members of the American Association of Swine Veterinarians; producers, represented by the National Pork Board (NPB) and animal health companies. Objectives 1 through 3 encompass Research, including research on vaccines-immunity (Objective 1), epidemiology-ecology (Objective 2) and host-genetics (Objective 3). Objective 4, Extension, is directed at the regional elimination of PRRS. Objective 5 encompasses Education and Outreach. In coordination with the NPB, which provides significant infrastructure support, the CAP is actively managed by a stakeholder board, project director, co-project directors and a diverse group of PRRSV research scientists.

Research projects support the collaborative efforts of multiple institutions and are funded for a total of four years. As part of a larger leveraging activity, the CAP returns four dollars for every dollar spent on research. The four projects currently funded include: Immunological consequences of PRRSV diversity (PI: William Laegreid, University of Illinois); Identifying ecologic and epidemiologic factors in the control of PRRS (PI: Jeffrey J. Zimmerman, Iowa State University); Positive prognosticators of immune protection and prophylaxis in swine herds (PI: Michael Murtaugh, University of Minnesota); and Characterization of host factors that contribute to PRRS disease resistance and susceptibility (PI: Joan K. Lunney, USDA ARS BARC). The latest tool in the control of PRRS focuses on the host genome. The PRRS Host Genetics Consortium (PHGC) was formed as the means to find genomic markers and genes linked to disease susceptibility and resistance, including vaccine response and disease tolerance. The PHGC receives support from the NPB, PRRS CAP, USDA, companies and other countries. The experimental model incorporates the infection of nursery pigs with a highly pathogenic laboratory isolate. Repeated measurements of virus load, weight gain and other phenotypic disease traits are collected over a 42 day period. To date, the PHGC has experimentally challenged almost 1500 pigs. SNP analysis of the first 600 pigs indicates that a limited number of genes are associated with two important clinical outcomes; decreased weight gain and increased virus load. The results indicate that selection of pigs with increased disease resistance is a real possibility and a new tool for PRRS control.

Regional elimination (PRRS CAP Objective 4) is directed by Robert Morrison, University of Minnesota. The CAP currently supports seven regional elimination projects, located throughout the Midwest. One tool for the integration of elimination projects was the creation of standard terminologies that can be used to communicate results across projects. With input and support from a terminology committee, Derald Holtkamp and others published the results in the paper "Standard Herd Classification and Related Terminology for PRRS Virus" (JSHAP, January 2011). The next step is to develop a common set of protocols that can be applied across projects. The resulting publicity has created an industry-wide interest in expanding regional elimination efforts.

A major success under Objective 5 of the CAP, Education and Outreach, is the support and organization of the annual International PRRS Symposium (IPRRSS). The symposium continues to grow and improve. In 2010, more than 270 participants from more than 19 counties attended the meeting in Chicago.

The PRRS CAP is a catalyst for several transformations within PRRS research community. Not only has the CAP achieved several key milestones in PRRS control and elimination, but is a model for how to attack large complex disease problems within the livestock industry.

L.9
AFRICAN SWINE FEVER UPDATE

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Key points about ASF

African swine fever (ASF) is one of the most complex and economically devastating viral infectious diseases of swine herds, producing great socio-economic impact in affected countries. ASF is listed as a notifiable disease to the World Organization for Animal Health (OIE).

African swine fever virus (ASFV) is a very complex and large enveloped DNA virus with a genome of 170-190 kbp. It is classified as a unique member of the *Asfarviridae* family, genus *Asfavirus* (1). This virus presents high genetic and antigenic variability, with 22 different genotypes described, all of them currently circulating on the African continent.
The natural hosts of ASFV are wild and domestic pigs of all breeds and ages. The virus also infects different species of soft ticks of the genus *Ornithodoros*, in which it can persist for periods longer than five years (2). Monocytes and macrophages are the main target cells of ASFV infection. No neutralizing antibodies are induced during infection, and no effective vaccine is available. ASFV in the environment and in infected materials is highly resistant to inactivation, surviving more than 15 weeks in putrefied blood or 1000 days in frozen meat (3).

ASF is a hemorrhagic disease that progresses with different clinical signs depending on isolate virulence, host, dose and route of exposure. Clinical signs in pigs and wild boars are very similar to those of other hemorrhagic diseases such as classical swine fever, salmonellosis or erysipelas; thus, laboratory diagnosis is required for differentiating between them. ASF clinical signs may vary from a hyperacute form with mortality rates of 100% from day 4 to 7 post-infection, to an asymptomatic, chronic form in some cases, which can lead to the presence of carrier animals. The presence of antibodies confers some protection, and it is associated with appearance of the chronic and carrier forms of the disease. European wild boars are usually more resistant than domestic pigs to ASFV infection, although they present a similar pathological and epidemiological pattern (4).

Chronic forms, carrier animals (wild or domestic) and infected ticks play an important role in the persistence and dissemination of the disease in endemic areas (7).

African wild suids such as warthogs (*Phacochoerus aethiopicus*), bush pigs (*Potamochoerus porcus*) and giant forest hogs (*Hylochoerus meinertzhageni*) are also infected by ASFV, but they usually do not exhibit clinical signs, allowing them to act as reservoir hosts in Africa (5). In some of these wild suids, ASF infection is characterized by low levels of virus in tissues and low or undetectable viremia (6). These levels of virus are sufficient for transmission to domestic pigs through tick vectors but usually not sufficient for transmission through direct contact between animals. This wild disease cycle makes it more difficult to eradicate ASF from eastern and southern Africa.

ASFV has been introduced into disease-free areas mainly by feeding contaminated pork products from international airports and seaports to domestic animals. Once established in domestic herds, infected pigs, carriers and pork products become the primary sources of virus dissemination.

Preventing the virus from entering disease-free areas is crucial and must be based mainly on preventing the introduction of potentially infected pigs or pork products, and on properly disposing of pork waste from aircraft and ships as well as other fomites.

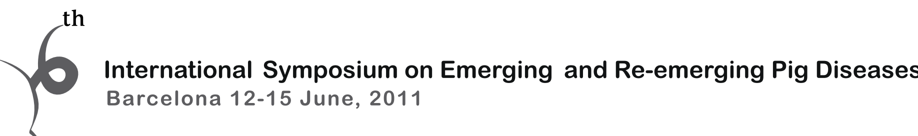
Control of the disease is based on early detection with rapid laboratory diagnosis and enforcement of strict sanitary measures (4). Laboratory diagnosis is essential to establish a correct diagnosis of the disease, due to the strong similarity of ASF clinical signs and macroscopic lesions with those of other hemorrhagic diseases of pigs. Several effective tests are available to detect infectious virus, viral antigens, viral DNA or specific antibodies induced by the 22 different ASF genotypes. The simultaneous detection of both antigen and antibodies in parallel is very important for establishing an effective diagnosis and evaluating the progress of the disease control program (4).

ASF eradication without vaccine is possible but difficult. The eradication of ASF from Portugal and Spain proved that vaccination is not required for eradication of this complex disease, even in endemic countries. However, the establishment of a good eradication program adapted to each specific scenario is essential (7).

ASF epidemiology

ASF was discovered by Montgomery in 1921 in Kenya. Since then, many sub-Saharan countries have been affected by the disease. During the 1970s and 1980s ASFV travelled around the world, affecting various countries in Europe, such as Portugal, Spain, Netherlands, France and Belgium, as well as some parts of the Americas, such as the Dominican Republic and Brazil. As a result of tremendous efforts, the disease was eradicated from all of these territories, but it persists on the Italian island of Sardinia and on the African continent, especially in the southeast.

During the 1990s and 2000s, the epidemiology and distribution of the disease changed. ASFV spread to other regions not typically affected by ASF. These included West African countries, where the virus was first reported in Nigeria (1997), Togo (1997) and Ghana (1999), as well as some islands, such as Madagascar (1998) and Mauritius (2007). The most recent change in the epidemiology of the disease occurred in 2007, when ASFV was reintroduced onto the European continent, this time via Georgia.



This significant epidemiological change may have been caused by a combination of several factors, the most important of which is the increasing presence of ASFV on the African continent during the last 15 years, as the virus has invaded disease-free territories. This implies increases in the amounts of circulating virus, in the number of infected animals, and therefore, in the amount of contaminated pig products. A second important factor is globalization. Today, people, animals and products travel around the world within short periods of time. The volume of animals, persons and products being transported increases day by day. The third important factor is the global financial crisis that has forced small farmers to meet their needs in new ways, such as by using swill or garbage to feed their animals.
These three factors, together with the resistance of ASFV in the environment and meat products, the presence of asymptomatic carrier animals, and the lack of a vaccine may help to explain how the disease has recently spread to several new territories.

ASF status of the Caucasus region and Russian Federation

In April 2007 a new outbreak of ASF genotype II, compatible with the virus circulating in Mozambique, Madagascar and Zambia, reached the European continent via Georgia. This ASFV is thought to have come from international ships that contained infected swills used to feed pigs near the port of Poti (8). After this introduction, the disease spread very quickly, affecting four different countries: Georgia, Armenia, Azerbaijan and the Russian Federation. Since the introduction of the virus in the Caucasus region, the OIE has been notified of more than 260 outbreaks, in which 76,000 animals have died. The disease and economic losses in the Russian Federation have been estimated at 25-30 billion RuR (0.8-1 billion USD) (9).

All the ASFV isolates found in the Caucasus region and Russian Federation since virus introduction show identical sequences, suggesting only one virus introduction in 2007 (10). Two recent outbreaks, in October 2009 and December 2010, occurred in areas very near European Union borders, less than 150 km away from Estonia and Finland (11).

The likelihood that ASF will become endemic and spread to nearby unaffected areas of the Russian Federation has been estimated as very high (12), due to the presence of key factors in the area: demonstrated infection of ASFV in wild boar populations, extremely high volume of illegal trade of pigs and pork products within the country, a traditional custom of swill feeding, absence of adequate veterinary services and lack of pig production infrastructures and traceability (13).
All these factors make control and eradication of the disease from this area very difficult, and increase the risk of spread to neighboring countries, especially those with commercial and socio-cultural relations with the Russian Federation. These observations are supported by comments of the Russian Chief Veterinary Officer, who recently predicted a spread of the disease toward the northern and northwestern regions (14). This situation could increase the risk of introduction into the EU.

What is happening in the EU and what is the risk of ASFV introduction?

The European Union (EU) is aware of the potential risk of ASFV introduction within its borders. The risk assessment (12) estimates the risk of ASFV introduction into the EU as moderate. However, this estimation, according with the evolution of the disease in Russia and the recent outbreaks near the EU border, should be reconsidered. The same risk assessment highlights the risk of introduction through contaminated products used for swill feeding. Historically, this was the most frequent route of ASFV introduction into disease-free countries, e.g. Spain, Netherlands, Belgium, Cuba, and, more recently, Georgia. This risk assessment further predicts that once the disease enters the EU, the risk that it will persist there is low, given the relatively high biosecurity of the pork production industry.
More detailed and complete analyses are being developed within the European project ASFRISK (EC, FP7-KBBE-2007-1, Project #211691) to estimate the most likely pathways, countries, and months for ASFV introduction into the EU. Preliminary results of this analysis place the likelihood of ASFV introduction into the EU by legal import of live pigs as low (15). This risk is mainly concentrated in Poland during the months of November and December, with the Russian Federation being the primary source of contaminated material. Methods and results obtained by this risk assessment may help to allocate financial and human resources in areas and periods at higher risk, helping to reduce the chance that ASFV will enter the EU.

Conclusions

- Circulation of the ASF virus has increased for the last 15 years on the African continent.
- The possibility that ASF will become endemic in the Caucasus region and the Russian Federation is very high.
- The risk of ASF virus introduction into the EU is increasing.
- An effective vaccine is not predicted to be available in the near future.
- An effective control and eradication program should be established for different scenarios and with the participation of all actors, such as veterinarians, farmers, and administrators.

Acknowledgments

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L.10

FOOT-AND-MOUTH DISEASE IN PIGS. CURRENT EPIDEMIOLOGICAL SITUATION

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Introduction

Foot and mouth disease (FMD) is a global problem, affecting productivity and trade where it is endemic, and requiring strong investments in preventing measures and surveillance where it has been eradicated.

According to the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals of OIE (2010) FMD is the most contagious disease of mammals and has a great potential for causing severe economic loss in susceptible animals.

Aetiology

FMD is caused by FMD virus (FMDV), which belongs to the genus *Ahtovirus*, family *Picornaviridae*. There are seven serotypes of FMDV namely, O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1. Infection with one serotype does not confer immunity against another.

Some FMDV strains have a pronounced predilection for one livestock species or another.

Susceptible species

All members of the order Artiodactyla (cloven-hooved mammals) can be infected by FMDV, as well as a few species in other orders.

Cattle are normally the most frequently involved in epidemics. Small ruminants, deer and buffalos may be naturally infected; however, they show little or none clinical signs. The disease is generally most severe in cattle and pigs.

Under certain conditions pigs may play an important role on the transmission of the agent.

Transmission

Pigs may become infected with FMDV by eating contaminated products, by direct contact with another infected animal, or by being placed in a heavily contaminated environment.

Probably the respiratory route is the more usual portal of entry for pigs, even if they may require as much as 600 times more than the exposure to aerosol virus required by a bovine or an ovine, to cause infection. On the other hand, pigs are much more susceptible to infection by the oral route than are ruminants.

Excretion of the virus can begin up to four days before the onset of clinical signs. This is of great epidemiological significance. The agent is excreted in large quantities in expired air, in all secretions and excretions and from ruptured vesicles. Pigs may liberate vast quantities of airborne virus in their expired breath, about 3000 times as much as cattle.
Once infection is established within a pig herd, transmission by direct contact between infected and susceptible animals can be very rapid, and many routes of viral entry may be involved. Maximum excretion of virus coincides with development of clinical disease and lesions on the snout, tongue and feet, and declines over the following 3 to 5 days as the antibody response develops.

Unlike ruminants that have recovered from FMD infection, pigs do not become carriers, and there is no evidence of viral ribonucleic acid persisting in infected pigs after 3 or 4 weeks of becoming infected.

Clinical signs and lesions

Incubation period in natural conditions varies with virus strain, the exposure dose and the route of entry. It may vary from 24 h to 11-14 days.

The disease is characterized by fever and vesicles on the feet, in and around the mouth, and on the mammary gland. In pigs the more severe lesions usually occur in the feet. Vesicles develop on the coronary band and in the interdigital space. Lesions at other sites are less frequent and less severe. Young pigs up to 14 weeks may die suddenly due to heart failure; piglets are particularly susceptible.

Incidence of disease in not immunized populations can be as high as 100%. Mortality rate in adult animals is usually negligible, but it can be extremely high in suckling piglets.

Diagnosis

In farms presenting high mortality of piglets and a significant proportion of pigs showing lameness, fever and vesicular lesions FMD should be strongly suspected. Actions should be taken immediately to secure a definitive diagnosis and prevent further spread on the agent.

In the laboratory, FMD can be diagnosed by virus isolation, detection of viral antigens, and serology.

Pigs may be affected by other vesicular diseases, which are clinically indistinguishable from FMD. Therefore, laboratory diagnosis of any suspected FMD case in pigs is therefore a matter of urgency.

Other pig vesicular diseases are:

1. Vesicular stomatitis (VS): may affect horses and cattle as well (sheep and goats may be experimentally infected). Two distinct immunological classes of VS virus have been recognised: New Jersey and Indiana. It is endemic in northern parts of South America and all Central America, being less frequent in USA. It is not present in other continents.

2. Swine vesicular disease (SVD): does not affect other species. It can be a subclinical, mild or severe vesicular condition depending on the strain of virus involved, the route and dose of infection, and the husbandry conditions under which the pigs are kept. Recent outbreaks of SVD have been characterised by less severe or no clinical signs; infection has been detected when samples are tested for a serosurveillance programme or for export certification. The last report to OIE from a European country was from Portugal in 2007: 1 farm was affected, with a population of 1800 pigs. All were eliminated by stamping out. The origin of the outbreak remains unknown.

3. Vesicular exanthema (VES): does not affect other species. It was originated in California, and became widespread in the USA during the 1950s, but a vigorous campaign to eradicate the disease was successful. In 1959, the USA was declared free of VES, and the disease was designated a foreign animal disease. It has never been reported as a natural infection of pigs in any other part of the world.

World epidemiological situation

During 2010, a total of 716 outbreaks of FMD were notified to OIE, affecting 21 countries from two continents: Africa and Asia. These records are the result of immediate notifications, meaning that cases occurring in endemic countries are not included.

Some data concerning those 716 outbreaks are presented in Table 1: the number of susceptible animals exposed to the virus, diseased, dead, destroyed and slaughtered animals. Source World Animal Health Information System, OIE, year 2010.

Table 1: data of 716 FMD outbreaks from 2010 notified to OIE and recorded in WAHIS

Parameter	Cattle	Small ruminants	Pigs
Animals exposed to virus	211,445	31,218	315,460
Diseased animals	11,999	20,091	13,954
Deaths	47	218	8,350
Destroyed	53,577	21,295	295,996
Slaughtered	95	662	182

Almost 14,000 pigs were directly affected, producing more than 8,000 deaths (estimated mortality rate 60%).

The 716 outbreaks were due to the following FMDV serotype: A: 14 (2%); O: 665 (93%); SAT1: 6 (1%); SAT2: 16 (2%) y Asia1: 15 (2%).

As a consequence of these outbreaks, near 300,000 pigs were destroyed. These are the direct cost of the disease. Economical losses in terms of international trade interruption and costs of control measures should be added to the direct costs in order to have a rough estimation of general losses.

The role of pigs in the epidemiology of FMD is not the same everywhere. Firstly, it strongly depends on the production system, in countries or areas having high density of pig farms the role of this species may be crucial. Secondly, it depends on the host tropism of the FMDV strain, for instance, the 1997 FMD epidemic that took place in Taiwan was due to type O/ Taiwan/97, which produced high morbidity and mortality in pigs, but did not affected cattle.

Measures of prevention

In FMD free areas or countries, import control including quarantine, is the first line of defence. Import quarantine policy should include pre-export testing and quarantine, animal heath certification and any necessary post-arrival inspection testing and quarantine. These policies should be based on the results of risk analyses. Border inspection posts (in airports, seaports and borders) should be able to properly intercept all risk products and animals. The swill feeding to pigs is a major way of introducing FMD into a country. Therefore, consideration should be given to banning swill feeding or at least implementing practices that will make it safe.

Measures of control

In both, endemic areas and areas that suffered reintroduction of FMDV, the control measures have the objective of reducing the reproductive rate (the average number of new cases produced by an infected individual in a time period). As for any other transmissible disease, those measures include three possible independent strategies:

1. Eliminating sources of infection: firstly, the sources of infection should be identified. Sources of infection are infected animals, contaminated premises, tools, vehicles, wild animals and others. Once identified, the sources of infection have to be eliminated by means of stamping out, slaughtering, disinfection, biocontention measures.

2. Interrupting contact between infected and susceptible individuals: movement interdictions, sanitary barriers, zoning, bioexclusion measures.

3. Decreasing proportion of susceptible animals: vaccination programmes.
None of these strategies is perfect. For that reason the simultaneous implementation of more than one strategy is convenient.

Both free and infected areas need to have in place an effective programme of early detection and rapid response. For early detection it is necessary: a. all actors involved in the animal production chain must be aware of FMD clinical signs and the procedures for notification; b. members of the official veterinary services have to be regularly trained, including simulation exercises; c. having a specialist FMD diagnostic team; d. laboratory diagnostic capabilities for rapid and certain diagnosis; e. access to a network of international reference laboratories; d. implementation of passive and active epidemiological surveillance programmes.

Due to the high transmissibility of FMDV, the control measures taken in endemic areas will have little probability of success if they are taken individually by a country or area. The control policies have to be undertaken at regional basis. For that, strong collaboration and transparency between parts are needed.

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L.11
INFLUENZA A VIRUS IN SWINE – MOVING BEYOND 2009

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Introduction

Surveillance for influenza A viruses (IAV) circulating in pigs and other non-human mammals has been chronically underfunded and virtually nonexistent in many areas of the world (1). This deficit is in spite of our knowledge that influenza is a disease shared between man and pig from at least as far back as the 1918 Spanish Flu Pandemic. In March-April 2009, a novel pandemic H1N1 emerged in the human population in North America (2) and demonstrated in a public forum the paucity of data on influenza viruses in swine. The gene constellation of the emerging virus was demonstrated to be a combination of genes from swine influenza A viruses (SIV) of North American and Eurasian lineages that had never before been identified in swine or other species. The emergent H1N1 quickly spread in the human population and the outbreak reached pandemic level 6 as declared by the World Health Organization on June 11, 2009. Although the 8 gene segments of the novel virus are similar to available sequences of corresponding genes from SIV from North America and Eurasia, no closely related ancestral IAV with this gene combination has been identified in North America or elsewhere in the world (3,4). Other than sporadic transmission to humans (5,6), swine influenza A viruses of the H1N1 subtype historically have been distinct from avian and other mammalian H1N1 influenza viruses in characteristics of host specificity, serologic cross-reactivity, and/or nucleotide sequence. The emergence of the 2009 pandemic H1N1 (pH1N1) virus brought a heightened awareness to the evolution and epidemiology of influenza A viruses in swine and presents a new era of challenges and opportunities for understanding and controlling influenza in pigs.

North American triple reassortant swine viruses. Swine influenza was first recognized in pigs in the Midwestern U.S. in 1918 as a respiratory disease that coincided with the human pandemic known as the Spanish flu. Since then, it has become an important disease to the swine industry throughout the world. The first influenza virus was isolated in 1930 by Shope (7) and was demonstrated to cause respiratory disease in swine that was similar to human influenza. The classical swine lineage H1N1 (cH1N1) derived from the 1918 pandemic was relatively stable at the genetic and antigenic levels in U.S. swine.

The epidemiology of IAV in pigs dramatically changed after 1998 when triple reassortant viruses containing gene segments from the classical swine virus (NP, M, NS), human virus (PB1, HA, NA), and avian virus (PB2, PA) (8) became successfully established in the pig population (9). The human lineage PB1, avian lineage PB2 and PA and swine lineage NP, M, and NS found in contemporary swine influenza viruses are referred to as the triple reassortant internal gene (TRIG) constellation (10) and the vast majority of the characterized swine viruses from the U.S. and Canada contain the TRIG, regardless of subtype.

After their emergence, the H3N2 viruses reassorted with cH1N1 swine IAV (11,12). Reassortant H1 viruses are endemic with the H3N2 viruses in most major swine producing regions of the U.S. and Canada. Since 2005, H1N1 and H1N2 viruses with the HA gene derived from human viruses emerged and spread across the U.S. in swine herds (13). The HAs from the human-like swine H1 viruses are genetically and antigenically distinct from classical swine lineage H1s. However, their TRIG genes are similar to those found in the TRIG cassette of the contemporary swine triple reassortant viruses.

To represent the evolution of the currently circulating North American H1 viruses, a cluster classification has been proposed. Viruses from the classical H1N1 lineage-HA evolved to form α-, β-, and γ-clusters based on the genetic makeup of the HA gene; whereas H1 subtypes strains with HA genes most similar to human seasonal H1 viruses form the δ-cluster (13). All four HA gene cluster types can be found with neuraminidase genes of either the N1 or N2 subtype. The HA from the δ-cluster viruses were shown to have most likely emerged from two separate introductions of human seasonal HA from H1N2 and H1N1 viruses and are differentiated phylogenetically by two distinct sub-clusters, δ1 and δ2, respectively (14). HAs of the δ-cluster were paired either with an N1 or N2 gene of human virus lineage and not of swine N1 lineage. The H1 SIV are evolving by drift and shift while maintaining the TRIG backbone and the resulting viruses differ genetically and antigenically with obvious consequences for vaccine and diagnostic test development (14).

Eurasian viruses. Swine IAV with genetic lineages that are distinct from the North American TRIG viruses evolved in Europe and Asia (reviewed in (15)). Although cH1N1 swine viruses previously circulated in Europe, Asia, and many other parts of the world, they were eventually replaced by a new lineage in Europe, a wholly avian H1N1 that emerged in 1979. The avian-lineage H1N1 was subsequently identified in Asia in 1993. Human-lineage H3N2 distinct from those in North America also emerged in Europe and Asia in the 1970s. Additionally, a human-like H1N2 emerged in pigs in Great Britain in the 1990s. A recent European surveillance study reported the continued circulation of avian-like H1N1, human-like H3N2 and human-like H1N2 in swine. All three subtypes were detected in Belgium, Italy and Spain, while only H1N1 and H1N2 viruses were found in UK and Northwestern France (16). The epidemiology of influenza viruses in Asia is complicated by the presence of North American and European lineage viruses, subsequent reassortant swine viruses between the two lineages, and reports of unique avian-lineage viruses. A complete description of IAV in Asian swine is beyond the scope of this paper.

Pandemic H1N1 (2009). The pH1N1 possesses a unique genome with six gene segments (PB2, PB1, PA, HA, NP and NS) with the closest known genetic lineage being the triple-reassortant influenza viruses of the North American swine lineage and the M and NA genes derived from a Eurasian lineage of swine influenza viruses (17). The 2009 pandemic influenza became infamously known as "swine flu" due to the phylogenetic origin of the gene segments. However, the unique combination of gene segments had never before been recognized in swine and since the recognition of the pandemic, the epidemiology in humans has not been affected by the subsequent human to pig transmission and outbreaks in pigs (17). The initial documented swine outbreaks were preceded by reported human influenza-like illness during the pandemic (18). The 2009 pH1N1 was shown to replicate efficiently in the lower and upper respiratory tract of experimentally infected pigs and to cause a clinical disease comparable to that typically observed during common enzootic influenza virus infection in swine (19-21).

Early reference to the 2009 pH1N1 as "swine flu" led to unnecessary alarm over the safety of pork meat products and culminated in the ban of exported pork from the U.S. by several countries, resulting in billions of dollars in lost revenue for the U.S. swine industry. However, contamination of fresh pork meat with the novel virus was experimentally excluded (22). Immediately after the onset in humans, cases of infection of pigs with the p2009 H1N1 were reported in different areas of the world. The first case was detected on April 28, 2009 in Canada in a farm with pigs that were not previously vaccinated against swine influenza (18, 23). Based on observations thus far, it is likely that the virus will continue to jump from humans to susceptible pigs with subsequent pig-to-pig transmission and establishment of yet another endemic virus in swine populations around the world. The 2009 pH1N1, a virus shared between people and pigs, has the potential to further change the epidemiology of influenza viruses in human and swine populations.

None of the 8 genes of the 2009 pH1N1 cluster tightly with the genes of SIV circulating in the U.S. prior to the outbreak in humans (3). In the phylogenetic analyses of each gene segment, the 2009 pH1N1 formed a distinct and independent branch from the U.S. swine lineage genes in viruses collected prior to 2009 and continues to do so. This suggests that neither the 2009 pH1N1 nor closely related progenitor viral genes were present in U.S. swine influenza viruses prior to 2009. A closely related progenitor virus with the same 8-gene constellation has yet to be identified in swine or other species, although a 2004 swine virus with 7/8 of the 2009 pH1N1 genome was identified in Hong Kong, China (3). The temporal gap between the closest known ancestor virus and the emergence of the pandemic virus in 2009 underscores the need for improved surveillance in animal hosts worldwide, as well as human hosts in underrepresented parts of the world.

A recent study demonstrated an enhancement of disease and pathologic changes in the lungs of pigs vaccinated with a virus with the H1 HA derived from human seasonal influenza virus (δ-cluster SIV) and challenged with 2009 pH1N1 (24). These data suggest that non-neutralizing inactivated vaccine-induced immune response contributed to the enhanced disease. This phenomenon has the potential to be realized in the swine population due to the concurrent circulation of genetically diverse H1 SIV among swine vaccinated with inactivated virus vaccines that are potentially mismatched to the circulating strains. The vaccine associated enhanced respiratory disease (VAERD) underscores the need for improved surveillance, antigenic mapping and vaccine strain selection. Additionally, this phenomenon may have relevance in the human population with some vaccine formulations as suggested by the association between the 2008-09 seasonal human vaccine and pH1N1 illness during 2009 (25) and low avidity, complement fixing antibodies in the lungs of fatal cases of pH1N1 in humans (26). Additional studies are in progress to further evaluate the kinetics and mechanism of VAERD in pigs.

Relevance to human health. The trivalent human vaccine no longer contains the seasonal H1N1 that circulated in the human population from the mid-1970s until 2009 due to its recent replacement by the pH1N1. If this remains the case in the coming years, the youngest subset of the human population may not have immunity against viruses related to the swine δ-cluster. The pig population may now serve as a reservoir of influenza genes historically shown to be successful in humans, such as the δ-cluster HA and NA; the pH1N1 HA, NA, and M; as well as the TRIG genes of human and pH1N1 virus lineage. This combined with sporadic infections with avian-lineage viruses in pigs may provide the right opportunity for continued IAV reassortment and emergence in pigs. The potential for further zoonotic transmission events of novel viruses from pigs to people remains an unknown but possible risk that must be considered.

Conclusions

Surveillance and genetic characterization of influenza viruses associated with respiratory disease outbreaks in pigs are necessary for monitoring the evolution of viruses in the pig population to minimally aid in the development of sensitive and specific diagnostic tests. In addition, antigenic characterization is critical to fully understand the relevance of genetic changes for vaccine strain selection, and vaccine efficacy must be evaluated minimally by serologic activity when new variants arise. The 2009 pH1N1 underscores the potential risk to human and animal populations of influenza virus subtypes and genotypes that may evolve with the SIV TRIG backbone and/or other virus lineages. Increased surveillance for the pH1N1 as well as reassortants between pH1N1 and endemic SIV in the swine and human populations is essential to understand the dynamic ecology of influenza A viruses in susceptible host populations.

The World Organisation for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO) formed OFFLU in 2005, a network of laboratories formally organized to demonstrate expertise in the animal health sector for surveillance, diagnostics, research, and control of highly pathogenic avian influenza H5N1. Influenza viruses circulating in swine and other animal hosts have recently been added to the OFFLU objectives and the potential for collaboration and exchange of information and resources between all influenza sectors is supported by OFFLU, with WHO also a contributing member. Although pigs may support the emergence of new viral reassortants, they may more often be the victim of cross-species transmission from people or birds than they are the source of new viruses. However, this cross-species transmission and the true directionality of virus movement

cannot be fully understood without surveillance. A global surveillance system in pigs has not yet come to fruition, despite the existence of several successful local and regional programs. A limitation of the regional approach is that the information is not always integrated and shared across species and regions, diminishing the effectiveness of surveillance efforts. Furthermore, unless a wide variety of pigs and geographical locations are sampled, the information may be biased and lead to inaccurate interpretation and/or decisions. The necessary global integration and sharing of data and resources for SIV will be addressed through the OFFLU network, but will require grass roots support from veterinarians and the swine industry. Facing the swine and human health issues with influenza proactively with science, transparency, and cooperation is our challenge and opportunity now and in the coming years.

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Oral communications

Porcine Circovirus

(O.01 - O.09)

O.01
OUTCOME OF PORCINE CIRCOVIRUS TYPE 1 (PCV1) INFECTIONS IN MID-GESTATIONAL PORCINE FOETUSES

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Introduction

It is accepted that PCV1 is nonpathogenic to pigs (1,2). However, PCV1 has been isolated from cases of congenital tremors and stillborn piglets (2,3). Up till now, nothing is known about the outcome of PCV1 infections in porcine foetuses. In the present study, virus replication and pathology were examined in porcine foetuses, inoculated with two PCV1 strains at 55 days of gestation.

Materials and Methods

Three conventional sows were submitted to laparotomy at 55 days of gestation. Three foetuses of each sow were inoculated: one foetus with PCV1 cell culture strain ATCC-CCL33; one with PCV1 field isolate 3384 (2) and one foetus with medium. Inoculations were performed by transuterine injection with $10^{4.3}$TCID$_{50}$ of PCV1 into the foetal peritoneal and amniotic cavities of the foetuses (4). At twenty-one days post-inoculation (dpi), the sows were euthanized and all foetuses were collected. All foetuses were examined for gross lesions and tissue samples from various organs were taken for histopathology, virus isolation and titration on PK-15 cells and for localization of PCV1 antigens by indirect immunofluorescence staining. DNA was extracted from organs of PCV1-inoculated and adjacent foetuses and PCV1 PCR was performed. In order to verify that the foetuses had been inoculated with the specific PCV1 strain, the full-length genome of PCV1 was amplified using heart and/or lung tissue from infected foetuses followed by sequencing. Abdominal fluids were tested for the presence of PCV1-specific antibodies by IPMA.

Results

All 6 PCV1-inoculated foetuses had a normal external appearance (Fig.1.a). Microscopic lesions include severe haemorrhages in the interlobular regions were observed in the lung tissues of two foetuses inoculated with PCV1 strain, CCL33 (Fig.1.b). Microscopic lesions were not present in the other four PCV1-inoculated foetuses. Gross and microscopic lesions were not observed in mock-inoculated and non-inoculated foetuses.

High PCV1 titres ($10^{2.9}$, $10^{4.6}$ and $10^{4.7}$ TCID$_{50}$/g tissue) were found in the lungs of CCL33-inoculated foetuses. All other organs were negative (<$10^{1.7}$ TCID$_{50}$/g tissue) by virus isolation. All collected organs from 3384-inoculated foetuses were negative by virus isolation. PCV1-positive cells (28 to 121 for CCL33-inoculated and 1 to 13 for 3384-inoculated foetuses / 10 mm^2 tissue) were observed in the lungs of all PCV1-inoculated foetuses (Fig.1.c). In general, heart, lungs, spleen, liver, kidney, thymus, tonsils and ileum of all PCV1-inoculated foetuses were positive by PCR. PCR and DNA sequencing recovered pure CCL33 and pure 3384 sequences from CCL33- and 3384-inoculated foetuses, respectively. No evidence for mixed samples (e.g. containing more than one PCV1 strain) was seen. All adjacent foetuses of PCV1-inoculated foetuses were negative by PCR. All mock-inoculated foetuses and their adjacent foetuses were also negative by PCR. All PCV1-inoculated foetuses had a low anti-PCV1 Ab titre of 10 to 40, except one foetus inoculated with CCL33, which had a titre of 160.

Discussion

In this study, PCV1 strain CCL33 was found to be pathogenic to porcine foetuses inoculated at 55-days of foetal life. Severe haemorrhages were present in the lungs of CCL33 inoculated foetuses (Fig.1.b). These lesions were correlated with high titres (up to $10^{4.7}$ TCID$_{50}$ / g tissue) in the lungs. This study also shows that the lung tissue is the main target organ of CCL33. On the other hand, the field strain 3384 remained non-pathogenic to porcine foetuses. A high PCV1 load could be essential to induce pathology.

From this study, it can be concluded that cell culture PCV1 replicates efficiently and produces pathology in the lungs of porcine foetuses inoculated at 55-days of foetal life.

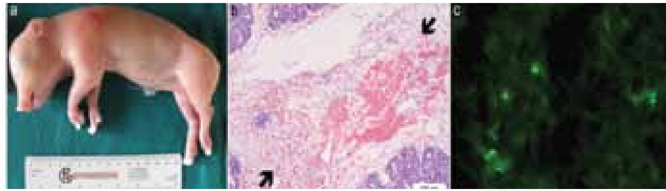

Fig. 1. Different aspects of PCV1 replication after inoculation of a 55-day old foetus. a) ATCC-CCL33 inoculated foetus without showing any gross pathology. b) Haematoxylin and Eosin staining of lungs of ATCC-CCL33 inoculated foetuses. Haemorrhages (indicated by arrow marks) in interlobular regions. Bar = 200 μm. c) PCV1-positive cells in the lungs. Bar = 100 μm.

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O.02

PRRSV ENHANCES PCV2a AND PCV2b REPLICATION AND DURATION OF SHEDDING

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Introduction

Several groups have characterized the shedding patterns of PCV2 from pigs experimentally infected with PCV2 and found that PCV2 is shed in nasal and oral secretions as well as in urine and feces (1). Horizontal transmission of PCV2 via oral-nasal and oral-fecal routes has been suggested as the main mechanism of infection (2) which was further confirmed by a recent experimental study (3).

Co-infections increase the severity of porcine circovirus associated disease (PCVAD) (4). Porcine reproductive and respiratory syndrome virus (PRRSV) infection has been shown to enhance the severity of clinical PCVAD under field and experimental conditions by enhancing PCV2 replication resulting in increased amounts of PCV2 DNA present in the sera of PCV2-PRRSV co-infected groups (4).

Co-infection of PCV2 and PRRSV in pigs is frequently observed in swine herds under field conditions; however, to our knowledge, the effect of PRRSV on PCV2 shedding patterns under controlled experimental conditions has not been investigated to date. The objectives of this study were to determine if co-infection of piglets with PRRSV and PCV2 has any affect on PCV2 shedding characteristics and if there are differences in shedding patterns between PCV2 subtypes (PCV2a and PCV2b) in co-infected pigs.

Materials and methods

Twenty-three, 2-to-6-week-old, PCV2 and PRRSV-naïve pigs were randomly assigned to one of five groups according to Table 1.

able 1. Experimental design.

Group	n	Inoculation	
		PCV2	PRRSV
Negative	3	-	-
PCV2a-I	5	PCV2a	-
PCV2a-PRRSV-CoI	5	PCV2a	PRRSV
PCV2b-I	5	PCV2b	-
PCV2b-PRRSV-CoI	5	PCV2b	PRRSV

PCV2 inoculation was done using PCV2a strain 40895 and PCV2b strain NC16845. For PRRSV inoculation, PRRSV strain VR2385 was utilized. Blood was collected on a weekly basis and tested for presence of anti-PCV2 antibodies by a PCV2 ELISA (5) and for presence of PCV2 DNA (6) and PRRSV RNA by PCR. Oral, nasal and fecal swabs were collected in regular intervals from day post inoculation (dpi) 0 until dpi 70 and tested by quantitative real-time PCR for the presence of PCV2 DNA. All pigs were necropsied at dpi 70.

Results

Negative control pigs remained PCV2 and PRRSV negative for the duration of the study. All pigs infected with PCV2 became infected as evidenced by PCV2 viremia and seroconversion. There was a significantly ($p < 0.05$) higher load of PCV2a and PCV2b DNA in serum, oral swabs, nasal swabs and fecal swabs in pigs concurrently infected with PCV2 and PRRSV compared to pigs singularly infected with PCV2 further confirming that PRRSV enhances replication and shedding of PCV2. Moreover, PRRSV infection significantly ($p<0.05$) prolonged the presence of PCV2 DNA in serum and increased the amount of PCV2 DNA in oral and nasal secretions and fecal excretions in the later stages of infection between dpi 28 and 70. Shedding patterns were similar between groups infected with PCV2a and PCV2b, indicating that there was no PCV2 subtype-specific interaction with the PRRSV isolate used in this study.

Discussion

A higher quantity of PCV2a and PCV2b DNA was found in serum, oral swabs, nasal swabs and fecal swabs in pigs concurrently infected with PCV2 and PRRSV compared to pigs singularly infected with PCV2, further confirming that PRRSV enhances replication and shedding of PCV2. Moreover, PRRSV infection significantly prolonged the presence of PCV2 DNA in serum and several body secretions, thus emphasizing the importance of PRRSV control in order to reduce the PCV2 shedding and transmission in the pig population.

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O.03
DEVELOPMENT OF A NEW MARKER ELISA (BACUCHECK™) FOR COMPLI-ANCE TESTING FOLLOWING THE VACCINATION OF PIGLETS AGAINST PCV-2

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Introduction

Infections with type 2 porcine circovirus (PCV-2) and therefore, also, antibodies of the IgG sub-class are very common in pigs. In Germany it is assumed that >85% of pigs are positive. All age groups can be affected and the symptomatology is very complex (PMWS, PRDC, PDNS). As the virus also frequently acts as a co-factor or predisposing factor to other pathogens, the term PCVAD (PCV-2 associated disease) is therefore widely used. Total annual losses due to PCV-2 within the EU are estimated at 600 million euros.

In diagnostics, the detection of pathogens is mainly by PCR. A test for antibodies of the IgG sub-class is also suitable for detecting the early phase of a PCV-2 infection. However, it has been difficult to differentiate between vaccinal and field antibodies.

Within the last three years, vaccination during the 3rd/4th week of life has become established as an essential prophylactic measure against PCV-2. Thus, it is an important mark of quality for the pig breeder to be able to prove that his animals have been vaccinated successfully and in good time. This paper describes the development of an indirect verification system for antibodies following vaccination.

The antigen used by the vaccine Porcillis® PCV (Intervet) is a structural capsid protein encoded by ORF2 of the virus genome. The protein is produced in insect cells using a baculovirus expression system. On the basis that baculoviruses only affect invertebrates, and assuming that baculovirus antigen can only be present in pigs following administration of baculovirus antigen containing vaccines, antibodies to baculovirus could serve as proof of vaccination. Furthermore, if the specific antibody response is quantifiable, an adequate antibody titre may reasonably be claimed to indicate that vaccination has been carried out properly.

Materials and methods

For verification, an indirect ELISA was developed in collaboration with Labor Diagnostik Leipzig. A purified and concentrated baculovirus extract was prepared by Intervet's R&D and used as antigen for the coating of ELISA plates. Quantification was undertaken using Synbiotics Serelisa® PCV-2 Ab Mono Blocking ELISA.

Results

With samples from a controlled vaccination trial (Eisele, Palzer et al. 2009; [vaccination during the 3rd week of life]), it was shown that in animals vaccinated only with placebo (Diluvac Forte and liquid paraffin), Baculo Ab ELISA (BacuCheck™) detected levels significantly below the present cut-off of 0.3 (average <0.1), only reacting at a specific titre against PCV-2 of <600 (average 368). For the vaccinated animals, on the other hand, the BacuCheck™ ELISA showed average S/P-values of 0.8 and an average specific PCV-2 titre of 2498. No unvaccinated animal manifested a positive reaction to baculovirus antigen. The values were the same for samples taken in the 7th and 9th week of life.

In various field trials from the ongoing routine diagnostics of Synlab.vet, an S/P value of 0.7–0.96 in the Baculo Ab ELISA was reached in the 5th to the end of 10th week of life in vaccinated animals, with titres of 2000-4000 in the specific PCV-2 test. Variations were evident due to, for example, too early vaccination and incomplete dosing. In field trials of older animals (gilts, fattened pigs), there were also occasional reactions in the BacuCheck™ ELISA in unvaccinated animals.

Discussion

The test is being evaluated in further trials and in further studies. For the time being, it can be concluded that the combination of the detection of antibodies to baculovirus and specific PCV-2 titres is reasonably sufficient to confirm that vaccination has taken place in compliance with the recommended dose regime. Its use should be limited to pigs up to 12 weeks of age.

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O.04

QUANTIFICATION OF PORCINE CIRCOVIRUS TYPE 2 (PCV-2) TRANSMISSION BETWEEN PEN-MATES IN VACCINATED AND NON-VACCINATED PIGS

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Introduction

Vaccination has become a worldwide pivotal tool to tackle Porcine Circovirus type 2 (PCV-2) associated diseases. The available commercial vaccines have been shown to reduce significantly the mortality rates of nursery and fattening pigs, and reduce lymphoid lesions and viremia (1,2,3,4). However, to the best of our knowledge, the impact of the large use of vaccination in the pig population has not been evaluated. Given the observed reduction of viremia in vaccinated pigs, one might expect a significant reduction of PCV-2 transmission in vaccinated pigs. The aim of this study was therefore to compare the PCV-2 transmission rate in vaccinated and non-vaccinated pigs through the estimation of the basic reproduction ratio (R_0). R_0 is the average number of secondary infections occurring from one single infected individual in a totally susceptible population. It actually measures how infection spreads in a naïve population.

Materials and methods

Animals and experimental design

The experiment was carried out in our air-filtered-level-3 biosecurity-facilities with (64+8) 21 day-old SPF piglets free from PCV-2 and without maternal antibodies. Four independent rooms were used, each of them having 2 pens managed separately (solid partition between pens). Rooms 1, 2 and 3 housed vaccinated piglets whereas piglets in room 4 were not vaccinated. In each pen, 4 inoculated were mingled with 4 susceptible piglets. Eight controls were kept in a separate compartment. Piglets in rooms 1 to 3 received 1 ml intramuscular injection of Ingelvac® CircoFLEX® at day 0, i.e.15 days before inoculation. The inoculated piglets were injected at day 15 with 6ml (5ml intratracheal + 1ml intramuscular) of a PCV-2 suspension (10^5 $TCID_{50}$/ml). The PCV-2 status of the pigs was monitored twice a week until 42 days post-inoculation using real-time qPCR (5) in sera to assess the PCV-2 genome load. In addition PCV-2 antibodies were monitored with an ELISA test (6).

R_0 estimation

We used the method described previously (7) based on a SEIR (Susceptible, Exposed, Infectious, Recovered) model with a constant latent period E (infected but not infectious) of 8 days as shown in Andraud et al., 2009 (8). The infectious status of piglets was determined at each sampling time according to the PCV-2 genome load and serological status (7). Piglets with genome load lower than 3.10^4copies/ml were considered as non shedders as evidenced previously (8). Using this SEIR model, the number of new infections is $\beta SI/N$ and the number of recoveries αI, where β and α are the transmission and recovery parameters respectively, estimated with a maximum likelihood method. $R_0=\beta*1/\alpha$.

Results

PCV-2 genome load was significantly lower in vaccinated than in non-vaccinated pigs (>1 log reduction), both in inoculated and sentinel pigs. The incidence of infection was also considerably reduced in sentinel vaccinated pigs. As consistent results were found between non-vaccinated and a previous trial (7), datasets were merged for R_0 estimation.

Table 1: R0 estimations in vaccinated and non-vaccinated pigs

Non vaccinated		**Vaccinated**	
β = 0.30 [0.20-0.46]	1/σ=17.1 d [12.4-23.6]	β = 0.10 [0.06-0.17]	1/σ=14.3 d [10.4-19.8]
R0=5.2 [3.1-8.8]		**R0=1.5 [0.81-2.6]**	

R_0 was 5.2 [95%CI 3.1-8.8] and 1.5 [95%CI 0.8-2.6] respectively in non vaccinated and vaccinated pigs (table 1). The average durations of the infectious period ($1/\alpha$) were 17.1 and 14.3 days, respectively.

Discussion

PCV-2 vaccination significantly reduced PCV-2 transmission, decreasing mainly the transmission rate β (3 fold lower) and also the duration of infectiousness. The R_0 estimate in vaccinated pigs with a 95% confidence interval including 1 suggests the infection would spread slowly in a vaccinated population and probably, under certain circumstances, might fade out. This will further be investigated implementing these estimates in an infection dynamics model.

Acknowledgements

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O.05
A STUDY IN SPAIN OF THE AGE AT ONSET OF PCV2 INFECTION DETECTED BY ANTIBODY RESPONSE TO PCV2 VIRUS

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Introduction

PMWS was first diagnosed in Spain in 1997 (1). It most commonly affects to pigs between 2 and 4 months old, but is not exclusive to this age group (2). The greatest incidence of PMWS in Spain occurred between 1999 and 2003 (3), coinciding with the high prevalence of acute outbreaks. However, during recent years, the presentation of the disease has become more chronic and enzootic.

Diagnosis depends on internationally accepted herd case definition criteria, based on clinical appearance at herd level (compatible clinical signs and mortality) and pathology and histopathology (4). However, herd criteria are difficult to apply in enzootic scenarios, so other options are necessary to demonstrate PCV2 infection in a population. The detection of serum antibodies, while not constituting a diagnosis, is evidence of PCV2 infection. The use of seroprofiles provides quantitative information about MDA, the optimum timing of vaccination and, in the absence of clinical signs, an insight into the age at onset of infection. With the advent of PCV2 vaccines, the need for such data increased, and a huge number of serological tests were undertaken.

The aim of the present study was to summarize the results of PCV2 serology carried out in recent years and to determine the mean age at onset of infection.

Materials and methods

The study includes serology undertaken on behalf of Intervet Schering Plough at independent diagnostic laboratories in Spain in 2008, 2009 and 2010. Only longitudinal or transversal case control studies are included in this survey if animals had definitely not been vaccinated and the age at which samples were taken is absolutely certain. The seroprofiles which fulfil these inclusion criteria correspond to 294 farms and 10,595 serum samples. The test used in all cases was the commercial ELISA kit Ingezim® Circovirus IgG/IgM, which detects antibodies against IgG and IgM differentially, allowing the determination of the timing of PCV2 infection, thus:

- IgM values ≥ IgG values: early active infection;
- xIgM values < IgG values: active infection;
- High IgG values and negative IgM values: late or resolving infection of a convalescent animal.

IgM is detected first, appearing between 7 and 10 days after infection; IgG appears a bit later, between 12 and 15 days post infection. The age at onset of infection was defined as the age at which IgM positive values were first detected. On this basis, farms were divided into three categories: early onset (<9 weeks), normal onset (9-16 weeks) and late onset (>16 weeks). The mean age at onset and the percentage of farms in each category were compared between the years included in the survey. Statistical analysis was carried out using the Levene Test and Pearson Chi-Squared test (GLM, SPSS 15.0 for Windows).

Results

Table 1 summarizes the average age at onset of infection and the percentage of farms with early, normal or late infection.

Table 1.

	2008	2009	2010
Nº herds	30	111	153
Nº samples	1,165	4,537	4,893
Av age at onset (wks)	11.77	13.10	12.93
Early onset (%)	10%	7.2%	2.6%
Normal onset (%)	80%	81.1%	85.6%
Late onset (%)	10%	11.7	11.8

No statistically significant differences were found between the proportions of early, normal or late onset of infection between years, though the percentage of early infections tended to reduce over time. Nor were there any statistical differences between the mean ages at onset over the three years. The youngest age at which IgM was detected was 6 weeks (3 farms), and the oldest was 26 weeks (2 farms). On 16 farms infection presented later than 20 w of age.

Discussion

The results of this study in which the age at onset of infection on more than 80% of the farms fell between 9 and 16 weeks of age, coincides with those of other authors (5). Nevertheless, it is important not to underestimate the importance of the percentage of late infections detected. The fact that more than 10% of the farms studied showed the age at onset of infection to be over 16 weeks demonstrates the particular value of animals being protected throughout the fattening period. In optimizing preventive programs against PMWS, this clearly underlines the need to choose the vaccine offering the longest duration of immunity.

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O.06

PCV2 MATERNAL ANTIBODY, NOT VIREMIA, IS ASSOCIATED WITH AVERAGE DAILY GAIN IN AN UNVACCINATED FARM WITH MODERATE PORCINE CIRCOVIRAL DISEASE (PCVD)

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Introduction

A field study in a Canadian farm with moderate porcine circoviral disease (PCVD) affecting nursery pigs was performed with the objective of assessing relationships among porcine circovirus type 2 (PCV2) viral load, mortality, morbidity, and growth rate and a number of pig- and production-related risk factors. This report focuses on the predictors of average daily gain (ADG) from birth to 15 weeks of age.

Materials and methods

160 piglets were monitored from birth to 15 weeks in a 600 sow farrow-to-finish research farm with moderately elevated post-weaning mortality (nursery 5.4±2.3%, finisher 4.6%±1.9%) attributable to PCVD. Two high and 2 low birth weight (BW) piglets per litter blocked by parity and farrowing week were weighed at birth, and 3, 9 and 15 weeks of age. Available stillborns from each dam were tested for parvovirus (PPV) and PCV2 by RT-PCR. Total IgG was measured in day 1 piglet sera.

PCV2 assessments: Antibody (IMPA, FFN) and DNA (copies/ml) in serum were measured in blood collected from each sow at farrowing, and each pig at each time point. PCV2 DNA concentration was log (base 10) transformed and averaged over the four time points. Antibody titres at each time point were converted to log (base 2) mid-value titres (1).

General immune competence was assessed in heparinized blood collected at each time point using the Disease Resistance Assay for Animals (DRAA, Metadis Inc., Ottawa), a multihit assay that measures proliferative responses of PBMCs following stimulation with a panel of 5 mitogens, then aligns a pig's individual responses along 3 specific immune pathways (TCR/CD3-dependent, JAK/STAT, PKC-dependent) using Principle Component Analysis (PCA) (2).

Room temperature and relative humidity were measured every 60 sec. for 42 days starting the day of weaning.

Statistical analysis: Multilevel mixed-effects linear regression (XTMIXED) was used to assess the predictors of ADG from birth to 15 weeks (ADG_{015}; kg/d). Litter was included as a random intercept. Biologically plausible predictors were considered in the full model if $P < 0.2$. Significant variables were retained following backwards elimination if $P<.05$. Animals dying or missing data bleeding were excluded from the analysis.

Results

Neither PCV2 nor PPV DNA was detected in any stillborn. 1/160 piglets had a low but detectable level of PCV2 DNA in serum at birth (log_{10}=4.46 copies/ml) but tested negative at week 3. This piglet had below average BW of 1.05 kg (average 1.41±0.29 kg) but ranked in the 80th percentile for ADG_{015}. The body weight of all pigs that survived to week 3, 9 and 15 averaged 6.0±1.3, 28.1±3.7, 62.5±9.3 kg respectively. ADG_{015} was 581±86 grams/day. Birth weight (BW), average nursery temperature and humidity, immune competence score at week 15, and PCV IgG antibody titre at day 1 were significant predictors of ADG_{015}. BW had the largest effect ($P<.001$, β=0.102, 95% CI=0.066, 0.139) on ADG_{015}, followed by nursery temperature ($P=.003$, β=0.045, 95% CI=0.015, 0.075) and humidity ($P=.005$, β=0.040, 95% CI=0.012, 0.068). Pigs in rooms with average temperature and relative humidity above 24.5°C and 55.5% respectively had superior ADG_{015} than pigs in cooler drier rooms. ADG_{015} was positively related to week 15 DRAA scores specific to overall CD3-TCR complex stimulation ($P=.002$, β=0.025, 95% CI=0.009, 0.040). ADG_{015} was positively associated with passively acquired PCV2 antibody levels ($P=.005$, β=0.008, 95% CI=0.02, 0.013). Collectively, these 5 predictors accounted for ~220 g/d variation in ADG_{015}.

Discussion

ADG_{015} increased ~12.5 g/d for each 3-fold increase in passively acquired PCV2 titre. The effect of maternal antibody on ADG_{015} however, was small relative to the effects of BW, nursery temperature and relative humidity, and CD3/TCR-related immune competence score. Total IgG, fluorescence focus neutralizing (FFN) antibody levels, average PCV2 DNA concentration, or the presence or absence of PCV2 viremia at any time point were not associated with ADG_{015}.

Acknowledgements

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O.07
IMPACT OF MATERNALLY DERIVED IMMUNITY AND EARLY PCV2 VIRAEMIA IN A UK COMMERCIAL HERD

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Introduction

Porcine Circovirus type 2 (PCV2) is a ubiquitous DNA virus and is involved in several diseases. PCV2 is known to infect pigs early on in their life, before weaning. The objective of this study was to investigate if the presence of maternally derived PCV2 antibodies (MDA) prevents early viraemia.

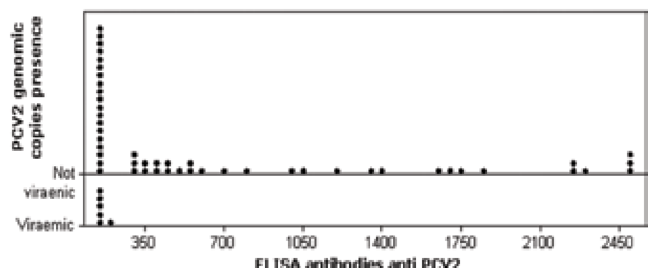
Materials and Methods

This study took place in a 250-sow 3-week batch farrowing, farrow-to-finish, PRRS and EP positive indoor sow herd. PMWS wasn't clinically present and no PCV2 vaccination takes place routinely in this unit. The studied unit broke with PMWS in 2000 and a piglet PCV2 vaccine has been used in 2010 without perceived benefits. It was decided to investigate if PCV2 was circulating during the pre and post weaning stage, the importance of MDA and necessity for PCV2 vaccination. Colostrum of 13 sows was collected up to 6 hours post farrowing. 112 piglets were individually identified with ear tags, weighed at birth, weaning and entry into the finishing stage and blood sampled at the two later time points. Blood samples and colostrum were tested by PCR and ELISA for PCV2 genomic copies and PCV2 antibodies respectively. Statistical analyses were undertaken using the Two-Sample T-Test, analysis of variance, Tukey simultaneous test and 95% Confidence Intervals. Tests were carried out using proprietary statistical software.

Results

PCR analysis of the sera at weaning revealed that 10 piglets (11%) were viraemic for PCV2 at weaning. Birth weight was significantly correlated with weaning and grower weight but didn't affect viraemia at weaning. A significant positive correlation ($r = 0.57$) existed between PCV2 antibodies in the colostrum and PCV2 antibodies at weaning ($p < 0.001$). Piglets with detectable levels of viraemia (PCR) at weaning had significantly lower levels of antibodies at weaning ($p<0.01$) than piglets not viraemic (Fig 1). Piglets born from sows with a high level of PCV2 antibodies in the colostrum had significantly higher level of antibodies in their sera at weaning ($p<0.01$) and piglets with lower levels of PCV2 antibodies at weaning are more likely to be viraemic ($p<0.01$). The weaning weight of the pigs with or without viraemia was 6.7 kg and 7.7 kg and 38.2 and 41.0 kg at 27 and 82 days of life respectively. Statistical significance was not demonstrated due to the low number of animals in the study.

Figure 1: Distribution of the results of PCV2 PCR according to PCV2 antibodies at weaning ($p<0.01$)



Each symbol represents up to 2 observations. $p<0.01$

Discussion

The results of this study are in agreement with previous reports that passive immunity to PCV2 reduces viral replication and provides protection[1]. Exposure to PCV2 is known to occur very early in life[2], and previous studies[3] have shown that vertical transmission occurs more frequently than believed before. Sampling of newborn piglets would clarify if vertical transmission occurred in this case. Previous studies have also shown that vaccination of sows with CIRCOVAC® increased weaning weights[4] and this study shows this is possibly due to the control of early viraemia. In the present study a positive correlation between PCV2 antibody levels in the colostrum and at weaning was found, also piglets with viraemia had significantly less PCV2 antibodies at weaning. This study has shown that piglets with poor passive immunity and born from sows with low level of PCV2 antibodies in the colostrum are at a higher risk of early viraemia.

Acknowledgements

We would like to thank George Gettinby's statistical analysis of the data.

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O.08

IMPACT OF SOW VACCINATION REGIME ON THE MATERNALLY DERIVED ANTIBODY TITRES IN 3 WEEKS OLD PIGLETS

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Introduction

Although first linked with clinical disease in 1997 (Postweaning Multisystemic Wasting Syndrome, PMWS, 1), porcine circovirus type 2 (PCV-2) was identified in archive samples nearly three decades before the first description of the disease (2). Infection is today considered ubiquitous among domestic and wild pigs, however, little current information on PCV-2 maternal antibody levels in domestic piglets is available. This absence of information is relevant given the widespread use of PCV2 vaccination in sows, and the potential influence of MDA on vaccine up-take in piglets. The objective of this investigation was to determine the maternally derived antibody (MDA) levels against PCV-2 in 3-week old piglets prior to vaccination in relation to established European vaccination practices.

Materials and methods

The study was conducted in Denmark, France, Germany, Italy, and Spain, with 51 herds tested in total, from which 297 sows and 1,827 piglets were selected. Piglets were selected from four groups of sows/gilts namely (a) sows/gilts having received no PCV2 vaccinations, (b) sows/gilts that received PCV2 vaccination as a piglet only, (c) sows/gilts that received PCV2 vaccination as a piglet and adult and (d) sows/gilts that received vaccination as an adult only. Wherever possible, 6 sows (2 gilts, 4 sows) were selected from each farm and enrolled together with 6 piglets from each sow. Blood samples were collected from each sow and all piglets at enrolment. Three farms were targeted from each category (a-d) in each country. Piglets were of approximately 3 weeks of age (21 ± 3 days) and had not been cross-suckled. Farm data was collected by means of a questionnaire. Serum samples were tested in a commercial PCV-2 antibody ELISA (Serelisa PCV2 Ab Mono Blocking, Synbiotics) as recommended by the manufacturer. Antibody levels were categorised as “High” or “Medium low” according to published thresholds (3, 4).

Results

Nine-hundred and thirty-seven of the 1,825 piglets tested had high PCV2 MDA levels. A significant positive association, between sow and piglet titres, was found (correlation coefficient 0.715, $P<0.0001$) indicating that as sow titres increased, piglet titres increased also. A regression analysis showed sow titre to be a significant predictor of piglet titres (R^2=0.51; $p<0.001$) This association was improved by including the vaccination status of the sows in the statistical model (R^2=0.53; $p<0.001$), so both factors should be taken into account when determining likely piglet MDA levels.

Further influences on piglet titre are being investigated and will be reported in the presentation.

Discussion

The initial analysis of the data highlights two key points: (I) there is direct association between sow titres and piglet MDA levels and (II) this association is improved by including the sow’s vaccination regime in the statistical model. This information is important to optimise piglet vaccination regimes on farms where high PCV2 MDA levels may interfere with early vaccination; piglet vaccination on farms with high sow antibody titres (particularly in farms where sow vaccination is practiced) could be delayed to avoid the interference of the high MDA levels with the vaccination. However, there are other potential influences on piglet titre, which are being investigated through a full risk factor analysis of this dataset, this analysis is on-going and will be included in the presentation.

Acknowledgements

The authors would like to thank the participating farms and veterinarians for their support. This work was funded by Pfizer Animal Health.

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O.09
FIRST REPORTED EFFICACY OF A SUB-UNIT PCV-2 VACCINE IN AUSTRALIA

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Introduction

Porcine circovirus associated disease (PCVAD) is a major cause of production loss for pig herds globally.

The main etiological agent is porcine circovirus type 2 (PCV-2) and vaccination has been shown to be effective in the control of PCVAD (1). The objective of this study was to evaluate the effect of a sub-unit PCV-2 vaccine which recently became available in Australia. Ingelvac Circoflex® was used at weaning to determine it's effeccts on growth performance, number of runts and post weaning mortality of clinically infected pigs growing under Australian field conditions.

Materials and methods

The study was conducted in a commercial 1,000 sow farrow to finish unit in New South Wales, Australia. The herd is vaccinating for both *M. hyopneumoniae* and *A. pleuropneumoniae.* The clinical symptoms of elevated morbidity and mortality due to multiple co- infections had been present in growing pigs for a period of two years before the study was carried out. PCV-2 had been diagnosed by IHC from tissue samples collected prior to the field study by the consulting veterinarian. The clinical signs of PCVAD occurred from 10 weeks to 15 weeks of age.

The study involved two batches of weaned pigs in a co-mingled study. Piglets were weaned at three weeks of age and were allocated by size and sex into pens. Within each pen, alternate pigs were vaccinated with 1ml of Ingelvac Circoflex® vaccine and the remaining pigs left unvaccinated. All pigs were individually identified at weaning and weighed (+/- 0.02kg) on a digital platform scale.

Piglets were individually weighed at 93 days and 122 days post-weaning. Any mortalities during the study were recorded along with cause of death. The growth differences were analysed statistically with Statistica v9.0.

Results

At 93 days (93 d) post weaning, vaccinated pigs had a higher average daily gain (ADG) and grew faster by +53 grams and +48 grams per day than the control pigs in batches 1 and 2, respectively. In addition, at 122 days (122 d) post weaning, vaccinated pigs grew +54 grams and +41 grams per day faster than control pigs for batches 1 and 2 respectively.

Table 1. Weaning weight and growth performance on vaccinated and control pigs per batch.

	Batch 1		Batch 2	
	Vaccinated	**Control**	**Vaccinated**	**Control**
No. of pigs	234	233	296	277
Wean Weight (Kg)	5.23 [a]	5.18 [a]	5.56 [a]	5.52 [a]
Wean to 93d ADG (gpd*)	548 [a]	495 [b]	558 [a]	510 [b]
Wean to 122d ADG (gpd)	643 [a]	589 [b]	639 [a]	598 [b]

* gpd = Grams per day and different superscripts within the same row and within each batch indicates significance at P<0.05

Runt animals were defined as pigs growing at less than 25% of the mean of the group at 122 days. There were 11.8% of control pigs classed as runts compared to 7.0% of vaccinated pigs.

Mortality rates in the control group were 7.92% and 7.90% for batch 1 and 2, respectively. While the vaccinated group had 3.45% and 3.9% for batch 1 and 2, respectively.

Discussion

The results of this study indicated a significant growth improvement in pigs vaccinated with Ingelvac Circoflex® at weaning. The number of runts were reduced by -40.2% and equally important, the mortality was also reduced by -50.6% to -56.4% in the vaccinated animals. The clinical signs of PCVAD at the grower-finisher phase were also reduced.

This is the first reported positive effect of a PCV-2 vaccine against PCVAD in Australia.

Acknowledgements

All veterinary aspects of this study was done under the guidance by Dr. Phil Sharmann of Casino Vet Services.

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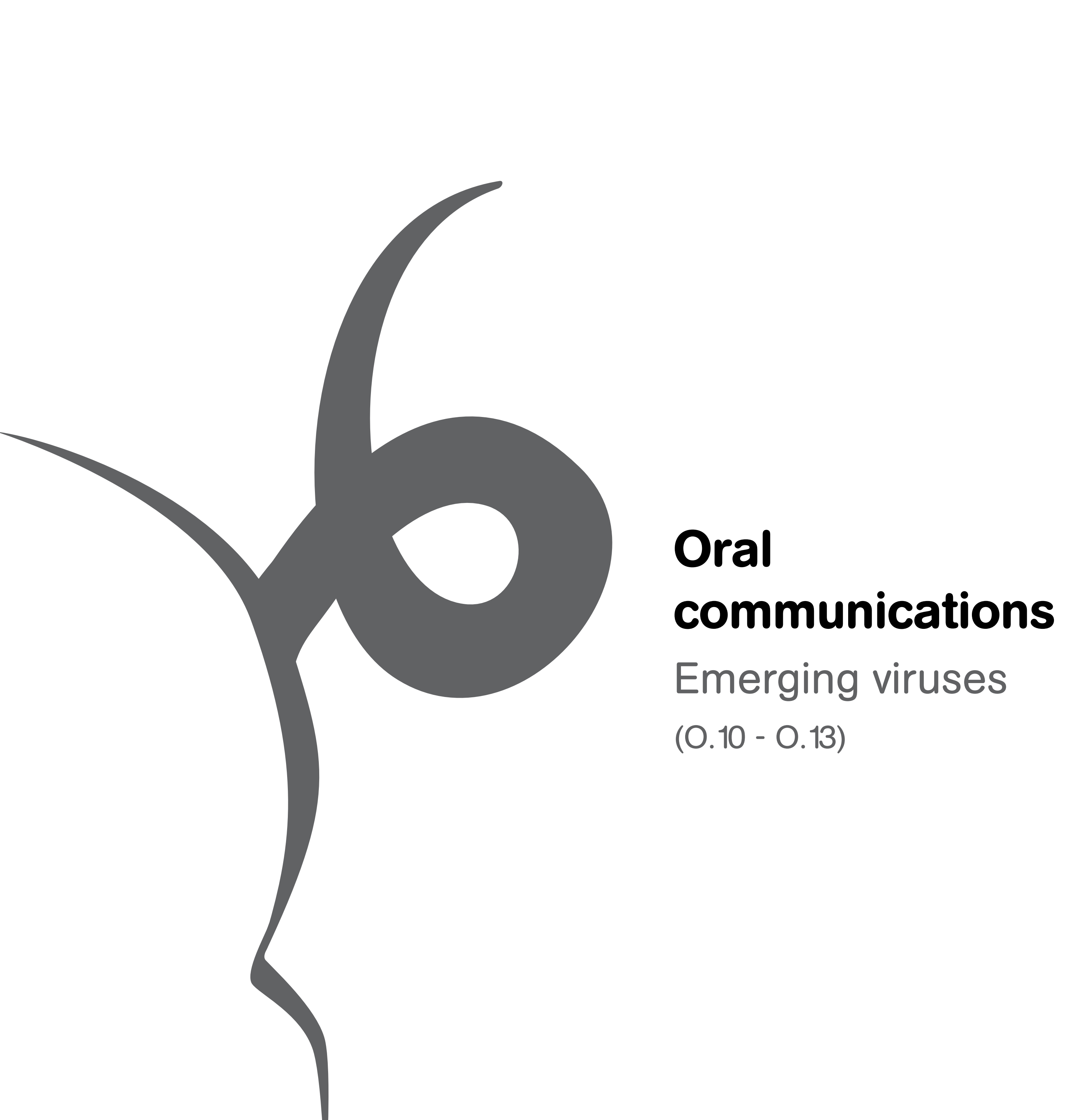

Oral communications

Emerging viruses

(O.10 - O.13)

O.10
PERI-WEANING FAILURE TO THRIVE SYNDROME (PFTS) DIAGNOSTIC INVESTIGATION TO IDENTIFY POSSIBLE INFECTIVE ETIOLOGIES

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Introduction

Porcine Periweaning Failure to Thrive Syndrome (PFTS) is a clinical condition characterized by anorexia, lethargy and progressive debilitation of pigs within 2-3 weeks of weaning. In affected populations, there is a striking contrast between clinically-affected pigs, which progress from having normal activity to lethargy within days of weaning, and unaffected cohorts, which grow and behave normally. The etiology and pathogenesis of PFTS are not understood, although several infectious agents have been identified in affected pigs. The objective of this report is to summarize the diagnostic investigation in a PFTS-affected farm with emphasis on identifying potential infective etiologies.

Clinical Presentation and Interventions

A well-managed, high health (PRRSV and *M. hyopneumoniae* negative; PCV2 positive) 100-sow farrow-finish farm experienced a 3.7 fold increase in nursery mortality beginning in 2007 (7.2% for 2007-2009; 1.9% for 2004-2006) almost exclusively due to PFTS. Weaned pigs show no evidence of residual illnesses from the suckling phase that could explain poor post-weaning health. Affected pigs are in good body condition at 21d weaning, and develop notable anorexia and lethargy within 7 days. Abnormal chewing or chomping behaviour is noted in a small percentage. Most die of progressive emaciation within 3 weeks. Management interventions targeting ventilation, piglet comfort, diet and water quality have not reduced PFTS-losses. PCV2 piglet vaccination and all antimicrobial strategies attempted have been unsuccessful. Losses have been partially mitigated following aggressive sanitation using hydrated lime or strong sodium hypochlorite solution.

Diagnostic Investigation

In response to the elevated mortality, 18 live pigs in poor body condition (PFTS) and 7 age-matched health pigs (HEALTHY) were submitted from the farm over a 30 month period. In addition, 4 healthy control pigs were submitted from each of 2 unaffected farms (CTR). Adjunct testing performed on appropriate tissues from selected pigs identified potential common swine pathogens. Results are presented in Table 1. No *Salmonella* spp., *Brachyspira* spp., *Campylobacter* spp., or *Helicobacter* spp. were identified in gut tissue. Testing of selected *C. perfringens* isolates confirmed type A (CpA).

Table 1. Pathologic and microbiologic results

	PFTS	Healthy	CTR
Pathology			
Thymic atrophy	15/17	0/7	0/8
Lymphocytic fundic gastritis	16/16	0/6	0/8
Jejunal villous atrophy	17/17	1/7	0/7
Colitis	18/18	5/7	1/8
Bronchopneumonia	7/18	0/7	0/8
Lymphoplasmacytic rhinitis	14/16	6/6	0/4
Microbiology (NT=not tested)			
C. perfringens	4/18	2/7	0/8
Pathogenic *E. coli*	8/12	2/3	4/7
AEEC[a] (histology)	8/18	3/7	0/8
PRRS	0/18	0/7	NT
Influenza A	0/18	0/7	NT
TGEV	0/18	0/7	NT
Rotavirus	4/18	0/7	NT
PCV2	0/18	0/7	NT
Enteric calicivirus	4/18	1/7	1/8
HEV[b] - tonsil	6/18	0/7	0/8
HEV - stomach, brain, intestine	0/18	0/7	0/8
PCMV[c]	17/18	7/7	8/8
Coccidia (histology)	6/18	0/7	1/8

[a]Attaching and Effacing *E. coli*; [b]Haemaglutinating encephalomyelitis virus; [c]Porcine cytomegalovirus

Discussion

CpA is normal intestinal microbiota in pigs. AEEC is typically of low pathogenicity and associated with other pathogens (1). Enteric calicivirus causes atrophic enteritis in gnotobiotic neonatal pigs, not in conventional or weaned pigs (2). Rotavirus causes villous atrophy and diarrhea. Diarrhea is an inconsistent sign of PFTS. HEV was detected only in tonsil, not in stomach, intestine or brain, and is unlikely causing clinical disease. PCMV rarely causes severe disease and no histological evidence of systemic infection was found. In conclusion, PFTS in the current farm is not caused by *Brachyspira*, PRRS, PCV2, influenza A or TGEV. HEV, PCMV, rotavirus, AEEC, *CpA,* enteric calicivirus and coccidiosis (probably *I. suis*) are present on the PFTS-affected farm, but signs do not match the reported clinical presentations for these pathogens.

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O.11

GLOBALISATION, GLOBAL TRADE AND POTENTIAL EMERGING PATHOGENS: THE EXAMPLE OF TORQUE TENO SUS VIRUS 1 AND 2

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Introduction

Increasing trade and global warming favours the emergence and re-emergence of diseases (1). Several of them have serious zoonotic concerns and global implications, and the biggest threat is seen as the outbreak of a pandemic event (2).

Torque Teno Virus (TTVs) are circular, single-stranded DNA viruses that infect several vertebrate species (3,4). In swine, *Torque teno sus virus 1* (TTSuV1) and *2* (TTSuV2) have been so far identified. TTSuVs are horizontally and vertically transmitted and lead to persistent infection (5). Natural selection and drift are the main forces shaping the evolution of TTSuV (6). Hence, the live pig trading would have the same effect as migration, increasing gene flow among populations.

The objective of this study was to analyze the effects of global trade of live pigs in the population structure and diversity of TTSuVs.

Materials and methods

Seventeen countries from different parts of the world were analyzed. From 358 PCR-positive samples, fragment of 678 bp (TTSuV1) or 719 bp (TTSuV2) of viral genome were amplified.

The impact of trade in the diversity of TTSuV1 and TTSuV2 was evaluated comparing the mean genetic distance matrices among countries with a distance matrix based on global trade statistics. This late matrix was constructed with the data available at the International Merchandise Trade Statistics (IMTS) of the United Nations comtrade Database (http://comtrade.un.org/), and the FAO Statistics Division (http://faostat.fao.org/).

Results

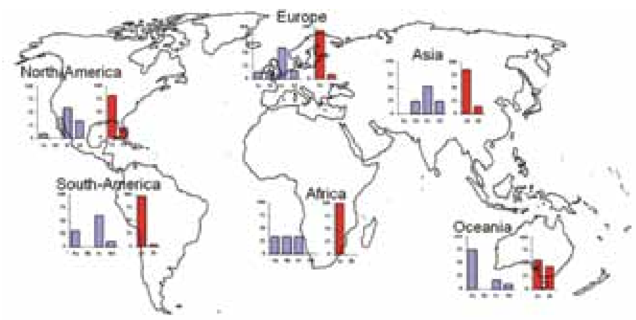
Four types of TTSuV1 and two of TTSuV2 were identified. The TTSuV types were differently distributed among countries (Fig. 1), and a small fraction of these differences had a geographic origin, both for TTSuV1 (2.66%) and TTSuV2 (4.37%). On the opposite, a significant relationship between genetic distance matrices with global trade statistics was reported.

Discussion

There is high amount of unidentified viral diversity that still remains to be uncovered, both in known and unknown viruses, highlighting the potential threats of unreported diversity in viral pathogens (7).

The restricted distribution of some TTSuV1 and TTSuV2 types may be related to historical isolation of pig populations. However, a larger fraction of the diversity is best explained by the exchanges of live pigs among countries, pointing to the direct relationship between movement of hosts and the diversity of their accompanying virus. The relationship between live pig trade and TTSuV diversity clearly illustrates the potential risk of global trade for food safety, a theoretical threat that has been repeatedly stated by the World Organisation for Animal Health.

Figure 1. World map showing the relative percentages of TTSuV1 (blue) and TTSuV2 (red) types for every continent.



Acknowledgements

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O.12
IDENTIFICATION OF AN ANTIGENETICALLY DIFFERENT PORCINE PARVOVIRUS (PPV) ISOLATE IN DENMARK

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Introduction

Porcine parvovirus (PPV) is a member of the family *Parvoviridae*. PPV is widespread in swine herds and causes reproductive failure, characterized by embryonic and fetal death, mummification, stillbirths and delayed return to estrus. PPV is recognized as an economically important cause of reproductive failure and vaccines to this virus are marketed worldwide to prevent such condition.

PPV contains a single-stranded DNA genome of about 5 kb. The structural proteins VP1, VP2 and VP3 are encoded by ORF2. VP1 and VP2 are a result of alternative spliced RNAs, giving VP1 a specific N terminus of 150aa.

Recent phylogenetic analyses of German PPV sequences revealed the existence of two PPV clusters (1). In this study we have analyzed Danish PPV isolates and genetic drift similar to that found for the German isolates was found. The results support the presence of two genetic clusters for PPV.

Materials and methods

The Danish PPV isolates came from reproductive failure cases submitted to the National Veterinary Institute, Denmark for diagnostic analysis. The 5 samples (1 from 2006, 1 from 2007, and 3 from 2009) were lung and liver tissue from aborted fetuses. DNA was purified by use of QIAamp DNA Mini kit. The Danish PPV isolates were sequenced as described in Shangjin et al. (2). Phylogenetic relationships were performed with the CLC DNA Workbench software using Maximum Likelihood Phylogeny (starting tree: UPGMA, substitution model: Jukes Cantor, rate variation included). Additional representative PPV sequences were retrieved from GenBank.

Results

The sequence analyses were performed on the first 1998 nucleotides of the VP1/VP2 gene. Sequencing of the last 264 nucleotides of VP2 gene for Danish PPV isolates is in progress. Nucleotide similarities among the Danish and representative PPV sequences were 98.2-99.8% and similarities among the Danish isolates were 98.3-99.4%. When analyzing amino acid sequences the similarities among all the isolates were 96.7-100% and 96.7-99.1% between the Danish isolates.

The phylogenetic analysis of VP1/VP2 PPV sequences showed two main groups or clades. One group contained the German sequences Tornau and IDT together with 4 of the Danish PPV sequences. The second main group could be separated into two branches. One of these branches contained European, American and Asian PPV sequences. The second branch of this second clade contained the German PPV-27a and one Danish PPV sequence.

The Danish PPV-2074 that group within the new German cluster show similarities of 99.8% and 100% for nucleotide and amino acid, respectively, to the PPV-27a strain. In addition, the Danish PPV-2074 contained the three amino acid substitutions (Q378E, E569Q, and S/P586T) which define this new distinct PPV cluster and all of these amino acids are exposed on the virus surface (1).

Discussion

The Danish PPV-2074 was isolated from a herd in which sows were vaccinated against PPV (Porcilis PPV, Intervet). Interestingly, based on sequence data the PPV-2074 isolate is antigenic identical to the PPV-27a strain. Previously, cross-neutralization studies of sera raised against strains included in commercial vaccines (NADL-2 and IDT) have showed low neutralization activity against PPV-27a strain, indicating incomplete protection (3). Furthermore, no protection against PPV-27a infection was found in a vaccine study using Porciparvac (IDT Biologika GmbH) (4). Unfortunately, the DNA sequence of the strain included in the Porcilis PPV vaccine is not available, however, the presence of antigenic diverse PPV strains in Danish pigs raises concern on the future protective effect of existing commercial PPV vaccines.

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O.13
INVESTIGATION OF THE PREVALENCE OF HEPATITIS E VIRUS CONTAMINATION THROUGH THE PORK FOOD SUPPLY CHAIN IN ENGLAND

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Introduction

Swine hepatitis E virus (HEV) infection is widespread in pig herds worldwide. Infected pigs excrete large quantities of virus in the faeces, and HEV RNA has been detected in retail pig liver in several countries. In addition to the liver, HEV RNA has been detected in extra-hepatic sites in the pig including stomach, small intestine, colon, spleen, kidney, lung and muscle (1). Although asymptomatic in pigs there is growing evidence for zoonotic transmission of HEV as a cause of autochthonous hepatitis E in developed countries, where it causes an acute choleostatic hepatitis, varying in severity from sub-clinical to fulminant. In developed regions, potential modes of transmission appear to include direct contact and contaminated food (raw / undercooked) (2). A recent investigation of risk factors for autochthonous HEV infection in Germany concluded that hepatitis E was likely a food-borne zoonosis (3).

The European FP7 project VITAL (Integrated Monitoring and Control of Foodborne Viruses in European Food Supply Chains) aims to gather data on virus contamination of food and environmental sources, for quantitative viral risk assessment. Fourteen European laboratories are investigating fruit, vegetable, shellfish and pork food supply chains for specified viral contamination. The VLA role was to investigate HEV contamination in the pork food chain in England.

Materials and methods

Samples were tested for the presence of HEV (target virus) and Porcine Adenovirus (PAdV; indicator of faecal contamination). Three points in the food supply chain were sampled, collecting faeces and liver samples at the production stage (abattoir), muscle samples at site of processing (meat processing plant) and sausage samples from point of sale. In addition, surface swabs were collected from these premises, in areas where viral contamination was considered more likely. Nucleic acid extraction and real-time PCR were performed according to standardised VITAL protocols. All samples were spiked with a control virus (Murine Norovirus) during nucleic acid extraction to demonstrate the extraction of amplifiable nucleic acid.

Results

PAdV DNA was detected at the production stage, and in a surface swab at point of sale. HEV RNA was detected at all three points of the pork food supply chain, and notably in 6/63 (9.5%) sausages tested at point of sale (Table 1).

Table 1: Prevalence of PAdV and HEV in the pork food supply chain

Point in chain	Sample type	PAdV DNA + / n (%)	HEV RNA + / n (%)
Production	Faeces	39 / 40 (98)	5 / 40 (13)
	Liver	6 / 40 (15)	1 / 40 (3)
	Surface swab	4 / 10 (40)	1 / 10 (10)
Processing	Muscle	0 / 40	0 / 40
	Surface swab	0 / 10	1 / 10 (10)
Point of sale	Sausage	0 / 63	6 / 63 (10)
	Surface swab	1 / 8 (13)	2 / 8 (25)

n, number of samples tested

Discussion

HEV was detected at all three points of the pork food supply chain, and with the exception of point of sale, the prevalence of HEV relative to PAdV would be consistent with a potential faecal contamination source. Six of 63 (9.5%) sausages tested had detectable HEV RNA: all six positive samples identified were from one of three batches of sausages that had been collected. In terms of foodborne transmission of HEV these represent the most significant findings.

Available data suggests that the consumption of raw / undercooked sausage is a potential route of HEV transmission, including transmission through the consumption of raw pork liver sausage (figatellu) in France (4), and the consumption of raw or undercooked pork meat or pork sausage in Germany, although this was not found to be a risk factor of autochthonous HEV infection in a case-control analysis (3). Crucially, it is not known if HEV that was detected in this study was viable and this will now be investigated using *in vitro* cell culture. Information on the viability of the virus will be critical in the assessment of the risk to public health of HEV contamination in the pork food supply chain.

Acknowledgements

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Oral communications

Porcine Reproductive and Respiratory Syndrome Virus

(O.14 - O.22)

O.14

NOVEL ASPECTS OF PRRSV VIRION COMPOSITION RELEVANT TO IMMUNITY AND PATHOGENESIS

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) emerged in the late 1980's in both Europe and North America as the causative agent of PRRS, now the most important disease of swine worldwide. Despite extensive characterization of PRRSV proteins by direct analysis and comparison to other arteriviruses, determinants of virulence, pathogenesis and protective immune recognition remain poorly understood. Thus, we hypothesized that the PRRSV virion contained additional features that contributed importantly to its biological characteristics. Procedures were optimized to obtain highly purified virions of the prototype type 2 PRRSV, strain VR2332, for physical characterization of structural proteins, analysis of the glycan shield, and screening for evidence of novel polypeptides. Key findings included discovery of a novel 51 amino acid polypeptide encoded in an alternative reading frame of the subgenomic mRNA encoding the major envelope glycoprotein, GP5. A similar ORF is present as an alternative reading frame in all PRRSV sgmRNA5 and in all other arteriviruses, suggesting that the protein, called ORF5a protein, plays a significant role in arterivirus biology. The N-termini of major antigenic targets, and glycosylation characteristics of envelope proteins also were elucidated. Our findings provide new potential targets for immunological and pharmacological intervention in PRRS.

Materials and methods

Type 2 PRRSV strain VR2332 was grown on MARC145 cells and harvested in media supernatant at 48 hr after infection. Virions were purified by precipitation with PEG8000 and banding in CsCl or iodixanol gradients. Purified virions were analyzed by SDS-gel electrophoresis, mass spectrometry methods, and responses to lectins that bind sugar structures on the virion. ORF5a protein was expressed in bacteria with myc and 6x-histidine tags, and chemically synthesized without modification. Antibodies were prepared to ORF5a protein in goat and rabbit.

Results

PRRSV particles produced by intact MARC145 cells consisted of a single population with a density of 1.23 mg/ml in CsCl. SDS-acrylamide gel analysis showed the presence of N, M and GP5 at the expected sizes as well as virion-associated cellular proteins. N-terminal amino acid sequencing indicated that mature GP5 is post-translationally cleaved between amino acids 31 and 32, so that a putative N-linked glycosylation site at amino acid 30 is not present in the mature virion. The amino termini of mature membrane protein, M, and nucleocapsid, N, are glycine and proline, at position 2 of the respective ORFs.

Additionally, a novel 51-aa polypeptide, ORF5a protein, was discovered (1). The ORF is present in sgmRNA5 at a position 10 bases upstream of the AUG start site of GP5 protein synthesis. Structure prediction suggests it is a single-spanning transmembrane protein with a short ectodomain and an ariginine and glutamine-rich endodomain. Its function has not been determined. However, it is highly conserved in PRRSV and an equivalent ORF is present as an alternative reading frame in all arteriviruses.

The carbohydrate shield on the surface of the virion consists of complex glycans rich in N-acetylglucosamine monosaccharides displayed on the ectodomain region of GP5. Lectins specific for N-acetylglucosamine blocked binding and infection of permissive cells.

Discussion

The specific protein and carbohydrate structures decorating the PRRSV virion surface are critically important for initiation of cell infection and evasion of immune defense. The discovery of ORF5a protein may reveal important targets of immunological protection. Genetic mutation of ORF5a in EAV severely attenuated viral growth, showing that the ORF is functionally important (2). Knowledge of the specific N-acetylglucosamine structures shows that alternative infection pathways may exist in permissive porcine macrophages compared to simian MARC145 cells.

Acknowledgements

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O.15
EARLY CONTROL OF PRRS VIRUS INFECTION

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Introduction

The critical issues of PRRS virus pathogenicity and virus/host interaction in vaccinated and naïve animals are still ill-defined, which may jeopardize the development of effective disease control strategies.

In general, there is evidence of inadequate response of the innate immune system during PRRSV infection, as well as of an abnormal delay in the onset of neutralizing antibody and interferon (IFN)-γ responses (1), which paves the way to prolonged virus persistence in the host. Therefore, other mechanisms account for cessation of viremia before the onset of the above features of adaptive immunity.

Owing to the above, we decided to perform an experimental infection in naïve pigs and to investigate different features of the innate and adaptive immune response related to the early control of PRRS virus infection.

Materials and methods

Twelve, 40-day old SPF pigs were allocated to two groups: 10 animals (group 1) were submitted to a type I PRRS virus infection by the endonasal route ($10^{5,3}$ $TCID_{50}$); 2 animals (group 2, controls) were inoculated with placebo (PBS). This procedure was repeated on post infection day (PID) 35 (homologous re-infection), and animals were sacrificed on PID 60. Pigs were clinically inspected and blood samples were analyzed at weekly intervals for PRRS virus RNA (RT Real-time PCR), interferon (IFN)-α, IFN-γ (sandwich ELISAs), IgM / IgG antibody (*LSI PRRS/SDRP–Lissieu, France*; *Herdcheck IDEXX Porcine reproductive and Respiratory Syndrome Antibody Test Kit*), serum-neutralizing (SN) antibody (bioassay on MARC-145 cells). The *in vitro* IFN-γ response to homologous PRRSV and IgA antibody in saliva samples were investigated as well. The frequency of both CD3 and non-CD3 IFN-γ+ lymphocytes in blood was determined by flow cytometry.

Results

Clinical dullness and slight anorexia with no respiratory symptoms were shown in the first week after infection. At necropsy, no lesions of the respiratory tract were evidenced, even though tonsils, pulmonary and inguinal lymph nodes were PCR-positive. All sera were also PCR-positive on PID 7, and negative (with one exception) on PID14. On PID 35, before re-infection, one pig only was still viremic, whereas no further viremic animals were detected later on. IgM and IgG serum antibody responses were detected by PID 14 with no major fluctuations later on. On the contrary, the saliva IgA response tended to wane after PID 14 and was transiently boosted in some subjects after re-infection. Little if any *in vivo* IFN-α response was detected in serum as opposed to IFN-γ, which showed a major peak on PID 7 associated to low IFN-α titres. On the contrary, with one exception, no PRRS virus-specific IFN-γ response was shown *in vitro*. The frequency of IFN-γ+, CD3+ T cells in blood showed minor fluctuations, as opposed to that of IFN-γ+, CD3-, non-T cells (NK), which increased dramatically between PID 0 and 7. All pigs showed SN antibody titres on PID 35; low SN Ab titres of two pigs were boosted following re-infection on the same PID.

Discussion

Our results indicate that early control of PRRS virus infection was not due in our study to a PRRS virus-specific immune response. A peculiar form of innate IFN-g response to virus infection was detectable on PID 7, which may have contributed to the subsequent decay of viremia. In turn, the onset of such a response was clearly associated to an expansion of CD3-, non-T IFN-g+ cells from PID 0 to PID 7. Interestingly, plenty of CD3+ T cells were also IFN-g + in flow cytometry on PID 0, in agreement with our study on constitutive expression of swine interferons (2). The above innate immune responses along with other undefined mechanisms caused early cessation of viremia, as opposed to the results accumulated in many other studies on PRRS virus infection of naive pigs. This can be probably traced back to fundamental properties of the PRRS virus strain employed in this study. This hypothesis is also supported by the demonstration of a SN antibody response 5 weeks after infection and by the B cell memory response observed in two pigs after PID 35.

Acknowledgements

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O.16
PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) IN SERUM AND ORAL FLUID SAMPLES FROM INDIVIDUAL BOARS: WILL ORAL FLUID REPLACE SERUM FOR PRRSV SURVEILLANCE?

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Introduction

Previous experiments used pen-based oral fluids to monitor porcine reproductive and respiratory síndrome virus (PRRSV) and porcine circovirus (PCV) 2 infections in wean-to-finish populations (1,2). The purpose of the present study was to determine whether oral fluid samples could be used to monitor individually-housed adult boars for PRRSV infection.

Materials and methods

In 3 trials, 24 boars, 5.5 months to 4 years in age, were intramuscularly (IM) inoculated with a modified-live PRRSV (MLV) vaccine (Trial 1), a Type 1 PRRSV isolate (Trial2), or a Type2 isolate (Trial 3). Oral fluid samples were collected daily and serum samples were collected twice weekly. Following the completion of the study, samples were randomized and blind-tested for PRRSV by real-time quantitative reverse-transcription polymerase chain reaction (qRT-PCR).

Results

With the exception of 2 boars in Replicate 2, all boars were successfully trained to provide oral fluid samples. A total of 2088 oral fluid samples were attempted. i.e., 29 days x 24 boars x 3 trials, and 1954 (93%) samples were collected. The average volume of oral fluid collected per boar across all trials was 17.9 ml (rang: 1 to 39 ml). PRRSV was detected in oral fluids at DPI 1 and all oral fluid specimens were PRRSV qRT-PCR positive at DPI 4. Although PRRSV was detected in both serum and oral fluid specimens through DPI21, a comparison of matched samples from individual boars showed that oral fluid was equal to serum for the detection of PRRSV at DPI 7 and more likely to be positive tan serum on DPI 14 and 21.

Discussion

Overall, oral fluid was superior to serum for the detection of PRRSV using PCR over the 21 day observation period in this study. The data showed that oral fluid offers distinct advantages over serum for the purpose of monitoring individually-housed animals for PRRSV infection using qRT-PCR. Advantages include easier simple collection, the ability to collect samples frequently (even daily) without incurring animal or employee resentment, the ability to select animals at random for sampling, and the cumulatively greater likelihood of detection in oral fluids vs serum. By extersion, oral fluid could replace blood sampling for monitoring PRRSV in other swine populations and in other application, such as PRRSV elimination/eradication programs.

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O.17
IMPACT OF SELECTED PARAMETERS ON EFFICACY OF PEN BASED ORAL FLUID COLLECTION FROM PIGS.

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Introduction

Oral fluid testing makes detection of microorganisms and antibodies in groups of pigs or individuals easier and cheaper to perform, compared to testing in serum. (1). Oral fluid testing is becoming important tool in diagnostic and epidemiological studies of PRRSV, PCV2 and SIV infections. However, sample collection requires understanding of pigs behavior as many of it aspects directly impact the yield of oral fluid. The aim of the present study is to analyse factors affecting the amount of oral fluid collected in standard pig herds in Poland.

Materials and methods

Table 1. Impact of presence of the straw, age of animals and temperature inside a pen, on average of quantity of oral fluid

Parameter		Number of pens	$\bar{x}$ of quantity of oral fluid/ pen
Floor	straw	172	1.73a
	slatted	362	2.64b
Age (weeks)	4-5	71	1.46a
	6-7	62	2.62b
	8-9	75	2.48b
	10-11	66	2.65b
	12-13	48	2.44b
	14-15	66	2.29b
	16-17	63	2.32b
	18-19	53	2.30b
	20≤	30	2.52b
Ambient temperature (2)	suboptimal	89	1.69a
	optimal	151	2.35b

- a, b: significant differences (p<0.005) between options within the parameter

Oral fluids were collected in winter (December 2010 and January 2010) from 24 farms. Totally 535 pens of 4 to 23 weeks old pigs were sampled. Presence of straw, ambient temperature in pens of 4-13 weeks old pigs as well as frequency of medication was noted in each farm and pen. From each pen one oral fluid sample was collected by hanging a piece of rope to which pigs had access for 30-60 minutes. The oral fluid soaked piece of rope was cut off and placed in a string bag and transported to the lab where oral fluid was recovered and it's volume measured.

Results

In 172 pens pigs were raised on deep straw or solid floor with bedding and in 382 on slatted floor. The mean yield of oral fluid was significantly higher (p<0.005) in pens with slatted floor than (2.64 ml vs. 1.73 ml). Of 240 pens where pigs 4-13 were housed in 89 the ambient temperature was suboptimal (2). The yield of oral fluid from such pens was significantly lower (p<0.005) than from pens with optimal temperature (1.69 ml vs. 2.35 ml) (Table 1). The pens with newly weaned pigs (4-5 week old) yielded significantly less oral fluid than pens with older pigs. No significant differences (p>0.05) were observed between pens with 6 to 23 weeks old pigs. From 23 pens no oral fluid was obtained. These included 18 pens with 4-5 weeks old pigs and 5 pens with 6-8 weeks old pigs.

Discussion

The results of this study clearly showed that the presence of straw and too low ambient temperature are the most important factors affecting the volume of the collected oral fluid. The impact of straw presence could be related with fact that it satisfy the pig's motivation for exploration so they are less willing to interact with hanging rope.

The impact of the age can be observed only in pigs shortly after weaning. From pigs of such age significantly lower yield of oral fluid was obtained. Also, from 18 pens with young pigs no sample of oral fluid was collected. From 6 weeks old and older no age impact could be observed. It could be related with restoration of hierarchical stability of group of animals inside a pen.

However, this is only a preliminary study and only several parameters were analysed, it is clear that the environmental conditions have to be considered while planning oral fluid collection.

Acknowledgements

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O.18
DIFFERENCES IN CROSS-PROTECTION BETWEEN TWO ISOLATES OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS THAT DIFFER IN THEIR CROSS-NEUTRALIZATION PROFILE IN VITRO

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Introduction

PRRSV isolates differ considerably in genomic, antigenic and pathogenic composition (1). Antigenic variability might account for the lack of cross-protection reported between PRRSV isolates. Because neutralizing antibodies (NAs) are known to play a role in protection (2), cross-reactivity of NA might be relevant in cross-protection. A previous study has shown that sera specific for different PRRSV isolates differ in their capacity to neutralize heterologous viruses (3). The aim of this study was to determine whether cross-neutralization *in vitro* is related to cross-protection *in vivo.*

Materials and methods

Two different studies were carried out. In Study 1, 54 two-month-old PRRSV seronegative pigs were immunized three times with an isolate that induces cross-reactive NAs. 4 weeks after the last immunization pigs were divided into ten groups, to which 3 seronegative age-matched pigs were added as controls. Each group was exposed to one of ten different PRRSV isolates (9 heterologous + 1 homologous). Blood was taken at selected times post-challenge (p.c.) to study NAs response (4) and viremia by virus isolation. All pigs were sacrificed 21 days p.c. and tonsils were collected to determine PRRSV presence by RT-PCR. Study 2 was carried out immunizing pigs with an isolate that induces only strain-specific NAs, but 45 animals were immunized and homologous isolate was not used for challenge.

Results

In both studies pigs developed detectable levels of NAs against the isolate used for immunization. However, when SN assays were performed using the isolate used for challenge, significant differences were observed between groups (Tables 1 and 2). Cross-reacting NAs were frequently found the day of challenge in pigs of some groups of Study 1 but were almost absent in most groups in Study 2.

Regarding virological parameters, all control pigs were viremic at all time points in both studies. Among immunized pigs, the number of positive pigs varied depending on the study and group considered (Tables 1 and 2). In study 1 viremia was not observed after homologous challenge or challenge with 5 of the heterologous viruses used and it was only sporadically recorded in pigs of the remaining 4 groups. On the contrary, viremia was frequently detected in pigs of all groups in Study 2, regardless of the virus used for challenge.

Table 1: Geometric Mean of NA titers against challenge isolates and number of viremic pigs at different times p.c (Study 1)

Group	Heterologous NA response			Viremia						
	Day 0	Day +12	Day +21	Day +3	Day +6	Day +9	Day +12	Day +15	Day +18	Day +21
1	0,25	2,70	5,35	3/5	3/5	1/5	4/5	4/5	2/5	2/5
2	0,10	0,82	4,13	3/5	5/5	3/5	5/5	4/5	0/5	0/5
3	0,18	3,57	3,52	5/5	5/5	4/5	4/5	4/5	4/5	3/5
4	0,10	2,93	3,76	4/5	5/5	4/5	3/5	2/5	1/5	1/5
5	1,64	4,48	6,88	0/5	1/5	2/5	4/5	1/5	0/5	0/5
6	0,10	1,32	5,14	5/5	3/5	2/5	3/5	3/5	3/5	2/5
7	0,10	1,41	4,64	4/4	3/4	3/4	2/4	4/4	2/4	2/4
8	0,16	4,64	6,63	5/5	0/5	1/5	4/5	2/5	1/5	1/5
9	0,72	4,99	5,56	3/5	3/5	2/5	4/5	3/5	2/5	1/5

Table 2: Geometric Mean of NA titers against challenge isolates and number of viremic pigs at different times p.c (Study 2)

Group	Heterologous NA response			Viremia						
	Day 0	Day +12	Day +21	Day +3	Day +6	Day +9	Day +12	Day +15	Day +18	Day +21
1	0,16	0,90	2,35	0/5	0/5	0/5	0/5	0/5	0/5	0/5
2	0,10	2,93	2,86	2/5	0/5	0/5	0/5	0/5	0/5	1/5
3	0,10	0,10	0,25	4/5	1/5	0/5	1/5	1/5	1/5	2/5
4	0,72	5,19	5,19	4/5	2/5	0/5	0/5	0/5	0/5	0/5
5	2,93	3,57	4,13	0/5	0/5	0/5	0/5	0/5	0/5	0/5
6	2,31	2,48	5,41	0/5	0/5	0/5	1/5	1/5	0/5	0/5
7	2,93	3,73	5,39	0/5	0/5	0/5	0/5	0/5	0/5	0/5
8	1,78	2,70	4,23	0/5	0/5	0/5	0/5	0/5	0/5	0/5
9	2,71	3,88	3,39	0/5	0/5	0/5	0/5	0/5	0/5	0/5

Discussion

The results of this study indicate that PRRSV isolates differ in their ability to induce cross-reactive NAs and protection against heterologous challenges, which implies that some isolates are better candidates for the development of new vaccines than others, based on their immunological properties. However, when we tried to correlate the titer of NAs against the challenge isolate with protection, we observed that, although there is a general tendency to find protection, at least against viremia, in groups of pigs that have detectable levels of cross-reactive NAs, while protection is less frequent in cases in which NAs are not detected, there are exceptions to this rule, and we were able to detect protection in pigs without NAs and viremia in pigs with significant titers of NAs. The role of other components of the immune response and the replication capacity of the isolates used for challenge might account for these discrepancies.

Acknowledgements

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O.19

PRRSV CHALLENGE OF LATE TERM PREGNANT GILTS VACCINATED WITH AN EXPERIMENTAL INACTIVATED VACCINE

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Introduction

In our laboratory, an inactivated PRRSV vaccine was recently developed. Vaccination of young pigs with the experimental vaccine resulted in a reduction of viremia after PRRSV challenge (1). In the present study it was examined if this vaccine can prevent transplacental virus transmission. This strategy may offer new perspectives for the control of PRRSV.

Materials and methods

Three gilts were vaccinated at 27, 55 and 83 days post-insemination (dpi) with 1 ml of the experimental inactivated vaccine based on the PRRSV Belgium A strain containing 10^8 $TCID_{50}$ before inactivation. Three gilts in the control group were not vaccinated. At 90 dpi, all animals were challenged with 10^5 $TCID_{50}$ homologous PRRSV. Blood was collected periodically until the end of the experiment. At 110 dpi, the gilts were euthanized.

Results

Antibodies were not detected by IPMA in any of the non-vaccinated gilts before challenge. Vaccinated gilts showed high IPMA antibody titres (9.5-11.5 log_2) week after the 2d vaccination (Figure 1). Non-vaccinated gilts remained negative for virus neutralizing (VN) antibodies during the entire experiment. Vaccinated gilts quickly raised VN antibodies at 3 days after challenge (1.0-5.0 log_2). In vaccinated gilts, viremia was detected earlier (3 days after challenge) than in non-vaccinated (5 days after challenge). The end point of viremia was reduced in vaccinated gilts (5/7/7 days post challenge) compared to non-vaccinated ones (10/10/>20 days post challenge). The percentage of PRRSV-positive fetuses in both groups was similar (42-46%).

Discussion

In the present study, a VN antibody response and a reduction of the duration of viremia in gilts was observed when vaccinated with a new inactivated vaccine. Despite this, the experimental vaccine was not able to prevent transplacental PRRSV infection. Even a short viremia during the last stage of pregnancy is able to cause transplacental infection. High titres of VN antibodies cause sterilizing immunity and blocks transplacental PRRSV spread if present before challenge (2,3). It is the purpose to activate the VN antibody response before 70 days of gestation. In this contest, vaccination of gilts with a live vaccine before insemination or during the early stage of pregnancy with subsequent boosting with the new inactivated vaccine may offer new perspectives for the prevention of PRRSV-induced reproductive disorders.

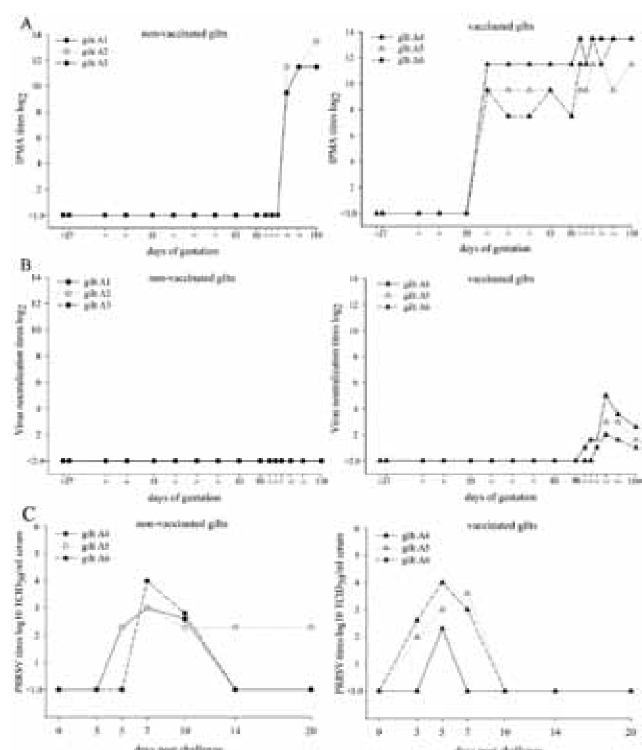

Figure 1. (A) PRRSV-specific IPMA antibody titers, (B) Virus neutralizing antibody titers and (C) PRRSV titres after vaccination and challenge. Three gilts were vaccinated at 27, 55 and 83 days post-insemination with an experimental inactivated vaccine. Three gilts in the control group were not vaccinated. At 90 dpi, all animals were challenged with 10^5 $TCID_{50}$ homologous PRRSV. Blood was collected periodically until the end of the experiment. At 110 dpi gilts were euthanized.

Acknowledgements

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O.20

CORRELATION BETWEEN GENETIC AND TEMPORAL DISTANCES, OWNERSHIP AND MUNICIPALITY FOR PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS STRAINS IDENTIFIED IN HERDS FROM QUEBEC, CANADA

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is known to be genetically highly variable; knowledge of this diversity within a geographical region or a production system may help to understand the epidemiology of the disease. The transmission of PRRSV between herds can occur over long distance by the introduction of infected animal or semen (1), and also over short distances by aerosols or various mechanical vectors (2). PRRSV strains sharing a high level of homology are suggestive of a common source of infection, and thus can be used to explore the temporal and geographical spread of the virus. The objective of our study was to determine the correlation between genetic and temporal distances, ownership and municipalities of PRRSV strains in Quebec.

Materials and method

As part of a PRRS surveillance project, samples were submitted by veterinarians and originated either from herds 1) experiencing reproductive or respiratory problems, 2) monitoring regularly their PRRS status, or 3) participating in a research project on risk factors for PRRS (3). RT-PCR and sequencing were done to obtain ORF5 gene. For wild-type strains, pairwise genetic distances of ORF5 sequences were calculated from nucleotides (Juke and Cantor model) in Bionumerics (Applied Math, inc., TX, USA) and temporal distance as the interval of days between the sampling date (SAS Institute Inc. Cary, NC, USA). Dichotomization was performed on all variables as shown in Table 1. Bivariate Mantel tests were computed in R (Vienna, Austria) among genetic and temporal distances, ownership and municipality. For significant correlations with genetic distance, the effect of adjustment for other distances was assessed using partial Mantel test. P-values were based on 999 permutations.

Results

A total of 1164 wild-type strains after exclusion of strains with missing information on one of the variables was gathered over an 11-year period (1999-2009). These strains were obtained from production sites belonging to 480 owners and located in 145 municipalities. Positive correlations were observed between the genetic and temporal distances, and belonging to different ownerships or municipalities (Table 1). Significant correlations were similar when adjustment was used for any of the other distances (partial Mantel test).

Table 1. Correlation coefficients (rm) between genetic distance (0:≥98%, 1:<98% homology) and other variables. Results from the bivariate Mantel test using 1164 PRRSV ORF5 sequences from 1999-2009.

Variable	Description of dichotomized variable	r_m
Ownership	Same (0) vs. different (1)	0.086*
Municipality	Same (0) vs. different (1)	0.068*
Time	<1 year (0) vs. ≥ 1 year (1)	0.062*

* Significant at p<0.001

Discussion

A correlation was observed between genetic homology and ownership suggesting that spread of a viral strain between production sites is favoured within an organization or a multi-site production system belonging to the same owner. It could be through a common source of animals (gilts, piglets or pigs), personnel or transport vehicles.

The genetic homology was also associated with the municipality of the site from which the strain was identified. This would suggest area spread either by vehicles, wildlife and domestic animals or aerosols. Unfortunately, the geographical coordinates (latitude/longitude) were only available for a small proportion of sites (<20%), precluding analysis of Euclidean distances which would have been useful to identify more accurately possible routes of transmission between herds.

Sequences identified at more than one year interval showed more genetic diversity than those obtained within a year, suggesting possible genetic evolution of the virus over the study period.

Our results suggest that many processes could be involved in the between-herd transmission of PRRSV at both short and long distances. Area spread and more specifically aerosols are often suspected to be responsible for a herd contamination. However, the role of a common source of infected animals, transport vehicles and personnel should not be underestimated.

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O.21
PORCINE REPRODUCTIVE AND RESPIRATORY SYNDORME VIRUS: AREA REGIONAL CONTROL AND ELIMINATION, THE NEXT LEVEL

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Introduction

The global swine industry faces economically devastating diseases such as porcine reproductive and respiratory syndrome (PRRS) (1), porcine circovirus associated diseases (PCVAD), and new variants of influenza. Consequently, veterinarians have developed different means for individual or groups of farms to control and possibly eradicate diseases. However, the risk of re-infection remains high even with the best current biosecurity practices (2). This situation has led to the consensus that more coordinated, or 'regional', approaches must be taken to combat PRRS and other emerging swine diseases, rather than isolated efforts on individual farms which are often frustrated by the reappearance of a disease. The objectives of this abstract are: 1. to present the methodology followed in PRRS area regional control and elimination (ARCE) projects in the United States (US), and 2. to report the advancement of these projects.

Materials and methods

Proposed working model used for PRRS area regional control and elimination projects:

- Phase 1: Feasibility study, objective: determine if the region's pork industry meets the minimal requirements to start a regional PRRS control and elimination project.
- Phase 2: Pig-Related site identification, objective: identify the general characteristics of the pork industry in the region.
- Phase 3: Region characterization, objective: determine PRRSV status, animal flow, and risk of becoming infected.
- Phase 4: Design PRRS control strategies, objective: design PRRS control strategies by site and neighborhood
- Phase 5: Execution and monitoring, objective: execute and monitor PRRS control strategies by site and neighborhood

Results

As of January of 2011, there are 10 PRRS area regional control and elimination (ARCE) projects in the US. Most of these projects are in either phase 2 or 3; and two of them are already in phase 5.

Fig. 1 ARCE projects in the US

Discussion

Our group considers PRRS area regional control and elimination a methodology to success, since it holds the potential to restore profitability to the swine industry. Momentum has been created in the US swine industry, and many other areas are ready to start ARCE projects. To date, we have identified four major components leading to the successful advancement of these projects: 1.- producer and veterinarian leadership, involvement and ownership, 2.- having a dedicated project coordinator, 3.- regular informational meetings with local producers and veterinarians addressing progress of the program, continuous education, issues related to confidentiality, and data access, and 4.- the establishment of a producer-paid economic fund to support, among others, diagnostic testing and salaries. If we are able to foster producers' participation, set aside individual interests and apply valid and developing knowledge on transmission that support effective rather than "psychological" biosecurity strategies, we will be able to efficiently assist our producers in the battle against PRRS.

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O.22

USE OF AIR FILTRATION AS A MEANS FOR REDUCING THE FREQUENCY OF PRRSV INFECTIONS IN LARGE BREEDING HERDS IN SWINE-DENSE REGIONS

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Introduction

Airborne transport of PRRSV has been documented to occur from 4.7-9.2 km (1,2). Based on this information, the filtering of incoming air has been proposed as a means to reduce this risk (3-6). To test this intervention, a study was conducted under controlled field conditions.

Materials and methods

The study involved 10 treatment herds and 30 control herds and was conducted over a 30-month period (Sep 2008-Feb 2011). Herds were selected utilizing published criteria (5,6). Specifically, a candidate herd was required to have a breeding herd inventory of 2400 sows or more, needed to be surrounded by four or more growing pig sites within a 4.7 km radius (1) and had to have experienced a minimum of three external PRRSV infections over the past four years despite the use of industry standard biosecurity practices. A summary of characteristics of study herds is provided in Table 1.On a monthly basis, treatment and control herds were assessed for clinical evidence of PRRS and blood/oral fluid samples collected from 30 piglets at weaning and tested by PCR. If positive, the ORF 5 region was sequenced and compared to historical PRRSV isolates.

Results

Over the 30-month study period, 8 treatment herds remained free of infection; however, 2 herds became infected due to documented breaches in transport and personnel biosecurity protocols. In contrast, 28 of 30 of control herds were re-infected with new variants. Of these 28 farms, 17 (62%) were infected one time, 7 (25%) were infected twice and 4 (13%) were infected three times. Chi square analysis indicated that treatment herds were significantly less likely to become infected when compared to control herds (p = 0.0001).

Table 1: Characteristics of study herds

Herd	#	# infected	BHI	Mean # sites/ 4.7 km	Mean # infections/4 years
Treatment	10	2	3163	8	5
Control	30	28	3238	8	5

Discussion

These results suggest that air filtration is an effective means to reduce the risk of external PRRSV introduction to large breeding herds located in swine dense regions. Studies are currently underway to continue to assess the sustainability of air filtration and to calculate its cost: benefit.

Acknowledgements

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Oral communications

Re-emerging viruses

(O.23 - O.26)

O.23
TOWARDS A DNA-VACCINE AGAINST AFRICAN SWINE FEVER: AN ONGOING EFFORT

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Introduction

African Swine Fever Virus (ASFV) is the causal agent of African Swine Fever (ASF), a fatal haemorrhagic disease for domestic pigs, producing subclinical infections in African wild pigs and also in ticks from the genus *Ornithodoros*, ASFV-natural reservoirs. ASF remains endemic in most countries from the sub-Saharan Africa where it causes a tremendous economical impact and in the island of Sardinia. This, together with the most recent ASF outbreak declared in the Caucasian region and in several Russian counties, opened new concerns about the potential spread of this disease to other European and Asian countries, including China (world's biggest pig-producer country). Unfortunately, there is no vaccine available against ASF, a notifiable disease to the OIE. Therefore, the only measure to control this disease relies on the early diagnosis, followed by the stamping out of all pigs within the risk area, a procedure not reliable nowadays, particularly in sub-Saharan Africa.

Materials and methods

Most of our vaccine strategies were based on the inoculation of naked plasmid DNA, encoding ASFV antigens. Aiming to enhance the immune responses induced, two main strategies were followed: i) Targeting antigens to professional antigen presenting cells by fusing them to either the APCH1 molecule (a single chain antibody driven against the SLAII antigen) or to the sHA; the extracellular domain of ASFV (a CD2-leukocyte antigen homologue), ii) Fusing antigens to ubiquitin, aiming to drive the encoded antigens to the proteasome, to enhance their SLAI presentation and the induction of CD8-T cell responses (CTL), as demonstrated before in mice against several disease models (1). Pigs were immunized intramuscularly with two doses (600 μg each) of: a) one only plasmid encoding three of the most immunogenic ASF proteins (p54, p30 and HA), or b) with multiple plasmids; ELI library immunization (2), using up to 4.000 plasmids covering the entire ASFV genome (170Kb in length), aiming to characterize new immunogenic antigens with vaccine potential. Pigs were finally challenged with a lethal dose of the E75 strain (10^4 UHA), two weeks after the last immunization. T-cell responses were followed by IFN-γ ELISPOT and specific antibody responses by ELISA, Western blotting and an infection inhibition assay (3). Protection was measured by survival rates and by measuring the viremia kinetics using and haemagglutination assay (3).

Results

Several lessons have been learned from the results obtained: i) Animal models lye in occasions and DNA vaccines working in mice can fail at inducing detectable responses and/or protection against ASFV when using pigs; ii) DNA vaccination encoding these same antigens can be exponentially improved by driving antigens to the APCs, albeit no protection was afforded against the lethal challenge; iii) Ubiquitination of same antigens has a dramatic effect both in the immune responses induced and in the protection afforded. 33% of the pigs vaccinated with pCMV-UbsHAPQ survived the lethal challenge and this correlated with the early expansion of specific CD8-T cells 3 days after infection; iv) Finally, addition of new antigens from the ASFV library to the plasmid cocktail, always as fusion with ubiquitin, significantly improved the vaccine coverage of our DNA experimental vaccines.

Discussion

The main line of research of our laboratory aimed to develop new vaccine strategies against ASFV. Albeit far from being optimal, our results have opened new avenues to finally obtain an affordable vaccine for the field in the near future.

Acknowledgements

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O.24

CLASSICAL SWINE FEVER VIRUS RESISTS IFN-BETA-MEDIATED PRIMING OF CONVENTIONAL DENTRITIC CELLS FOR ENHANCED IFN-ALPHA RESPONSE

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Introduction

Classical Swine Fever Virus (CSFV) is a monocytotropic virus that causes viral haemorrhagic fever (VHF) in pigs. A hallmark of the disease are the high IFN-α level found in the serum early after infection followed by an inflammatory cytokine storm with elevated levels of TNF-α, IL-1β and IL-6. The likely in vivo source of the IFN-α are plasmacytoid dendritic cells (pDC) representing the only cell type that produces IFN-α upon CSFV infection *in vitro* and *in vivo* (1). In primary target cells of the virus such as macrophages, conventional DC and endothelial cells the viral protein N^{pro} inhibits via the degradation of IRF-3 the induction of type I IFN and other cytokines *in vitro* (2-4). One explanation for the discrepancy between the unresponsiveness of primary target cells of CSFV *in vitro* and the increased levels of several cytokines found *in vivo* could be the particular environment to which the cells had been exposed to prior to virus infection. Since type I IFN is known to induce the up-regulation of many proteins that are involved in DC activation (like IRFs) or that are acting as viral sensors (such as RIG-I) we hypothesised that the early IFN-α response in the serum sensitizes the immune cells for enhanced responsiveness against virus infection and augmented pro-inflammatory cytokine response upon CSFV infection.

Materials and methods

Important target cells of CSFV such as bone marrow derived, GM-CSF induced DC or Flt3-ligand induced DCs, monocyte-derived DCs and pDCs were pre-treated with IFN-β and expression of the proinflammatory cytokines IL-1β, IL-6 as well as IFN-α was assessed after CSFV Alfort vA187 wild-type or N^{pro} mutant infection. As a control the influenza A virus (IAV) H5N1 NIBRG23 was employed.

Results

In general CSFV wild-type did not elicit a cytokine response in any cell type employed besides pDCs whereas N^{pro} mutant virus infection resulted in low IFN-α and IL-1β expression in Flt3L-derived DCs and robust IFN-a induction in enriched pDCs.

While IFN-β pretreated pDCs showed enhanced IFN-α responses induced by CSFV, non-pDCs could not be sensitized for increased IFN-α or pro-inflammatory cytokine expression upon CSFV infection. In contrast to this, all primed DC types showed augmented IFN-a responses following IAV- infection or polyIC stimulation. And IFN-β sensitized GM-CSF derived DCs showed increased IL-6 and IL-1β expression following LPS exposure.

Discussion

During both, influenza and CSFV infection, early pDC-derived type I IFN responses are found in vivo. These can sensitize the DC system for enhanced IL-6 and IFN-α responses. However our results indicate that IFN-β priming for enhanced cytokine secretion was dependent on the DC-type and the virus employed. The fact that CSFV-induced IFN-α expression were only primed in pDCs highlights the important role of pDCs in innate immune responses and disease pathogenesis.

Altogether, our results indicate that CSFV, in contrast to Influenza virus, has evolved to also escape IFN-sensitized cells, a puzzling observation considering the in vivo observed “cytokine storm”.

Acknowledgements

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O.25

IMPROVE TOOLS AND STRATEGIES FOR THE PREVENTION AND CONTROL OF CLASSICAL SWINE FEVER USING A LIVE RECOMBINANT VACCINE (DIVA)

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Introduction

Due to the vast economic consequences of classical swine fever (CSF) outbreaks, emergency vaccination plans are under discussion in European Union Member States. However, animals vaccinated with the conventional C-strain vaccine are subject to trade restrictions. To ease these restrictions, potent marker vaccines and accompanying diagnostic tools are required.

One vaccinal strain is the chimeric pestivirus CP7_E2alf. The results support CP7_E2alf as a promising marker vaccine candidate to control CSF in domestic pigs and wild boar. This abstract presents the preliminary tests, further studies are in progress

Materials and methods

Vaccine virus. CP7_E2alf virus is a chimeric pestivirus whose coding sequences for the major envelope protein E2 of bovine viral diarrhea virus (BVDV- strain CP7) are replaced by E2 of the CSFV strain Alfort187. The vaccine has been evaluated both for intramuscular (IM) as well as oral vaccination as a bait vaccine.

Challenge virus. Highly virulent CSFV strain Koslov (isolated in Czech Republic).

Animal experiments. Safety study on pregnant sows, ruminants and rabbits; Detection of CP7_E2alf and conventional C-strain vaccines in blood and tissue samples of pigs 42 days after vaccination; Comparison of CP7_E2alf and conventional C-strain vaccines after oral immunization in domestic pigs (1); Efficacy study on wild boars after oral vaccination against a challenge with CSFV strain Koslov; Efficacy study on domestic pigs after oral or IM vaccination against a challenge with CSFV strain Koslov; Onset of immunity after oral or IM Immunization; Dose titration in pigs vaccinated with a single dose of CP7_E2alf based on neutralizing antibody response.

Results

Safety:

- Safe. No significant adverse effects were observed in domestic pigs and wild boar. No effects on farrowing or the reproductive performance of the pregnant sows following vaccination were observed.
- The vaccine is safe after oral application in calves, lambs, goats and rabbits.

Efficacy:

- Protective immunity in domestic pigs and wild boars independently of the application route. Protection against clinical signs, leukopenia and viraemia (2, 3).
- Minimum protective dose has been defined (4).
- The onset of immunity is 1 week after intramuscular vaccination and 2 weeks after oral vaccination against a challenge with CSFV strain Koslov (4).
- Duration of immunity (DOI) after 1 shot has been evaluated based on neutralizing antibodies (NAbs). The animals have NAbs at least 128 days.

Discussion

The candidate live recombinant CP7_E2alf (DIVA) is an important tool to supplement or replace the conventional vaccines in the eradication program for classical swine fever.

Acknowledgements

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O.26
EXPERIMENTAL INFECTION OF WARTHOGS (PHACOCHOERUS AFRICANUS) AND BUSHPIGS (POTAMOCHOERUS LARVATUS) WITH CLASSICAL SWINE FEVER VIRUS

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Introduction

In 2005, classical swine fever (CSF) subtype 2.1 was diagnosed in the Western Cape Province of South Africa after 87 years' absence from the country and was subsequently confirmed in the Eastern Cape Province (ECP), widely populated by rural communities practising communal farming. Two indigenous wild suids; the desert warthog (*Phacochoerus africanus*) and the bushpig (*Potamochoerus larvatus*) are widely distributed throughout southern Africa with the characteristic valley bushveld of the ECP specifically harbouring large numbers of bushpigs.

The question arose whether these wild African suids are susceptible to infection by CSF virus, and if so, what pathological lesions will be observed. No data regarding these African wild suids' susceptibility to CSF were available.

Materials and methods

Seven sub-adult warthogs (all males) and six jeuvenile/sub-adult bushpigs (five females and one male) were captured and all but two of each species were infected intranasally with the 2005 Elsenberg (SA) isolate of CSF, in two separate experiments. For bushpigs, two animals were removed prior to challenge and reintroduced the following day. In the case of warthogs, two in contact (I-C) animals were left in the same pen for welfare reasons. Nasal and saliva swabs and blood were taken throughout, and surviving animals were killed after day 42, and studies were undertaken to determine presence of virus and antibody in these two hosts.

Results

The warthogs, which were clinically normal throughout the study, developed little in the way of clinical signs. The bushpigs, in contrast, developed overt clinical signs reminiscent of CSF in domestic pigs. Four bushpigs succumbed during the trail, with one dying before any clinical signs could develop.

Viral antigen in bushpigs was detected in serum from 4-10 dpi in inoculated animals and at 14 dpi in the I-C animals, with samples remaining positive for between 4-19 days. One bushpig remained antigen positive for 9 days whilst one in contact remained positive for 19 days when it was euthanised. In contrast, viral antigen was only transiently detected in serum of one of the inoculated warthogs between days 7 and 10 and one I-C warthog at day 17.

Viral RNA was detected in nasal swab samples from all of the inoculated bushpigs at dpi 4 or 6 and in saliva between 2 and 12 dpi. BP1, an I-C animal, had detectable RNA in a nasal swab at day 8, but was only consistently positive after day 14, until it died at day 22. The other I-C bushpig had RNA in both nasal swabs and saliva at 10 dpi. Once PCR positive, all excretions in an animal remained positive until until euthanasia.

For warthogs, all secretions from inoculated warthogs became PCR positive between 7 and 10 dpi and, in the case of I-C animals, intermittently from 10 dpi and consistently from 17 dpi. Once positive, viral RNA could again be detected for the remainder of the experiment, up to 42-44 dpi. Interestingly, for one warthog, viral RNA weas present in nasal swab samples from 7 dpi, whilst only one saliva sample was positive at day 42

Seroconversion was observed between 10-12 dpi in the infected bushpigs and at day 14 for the two in contact animals. BP 3 did not have any detectable antibodies, despite having viral circulating antigen present between days 10-19, after which it was euthanised. BP 6 died unexpectedly at 4 dpi and no antibodies had been detected at that time.

The infected warthogs seroconverted between 7 and 14 dpi, whilst the I-C animals seroconverted at day 17-21. All the warthogs remained seropositive for the duration of the study.

Intra-species transmission was verified in both cases by the two in-contact animals of the same species in each experiment, which also exhibited the clinical signs seen in the animals directly challenged.

Discussion

Bushpigs exhibited severe clinical signs upon infection, rapidly developed viraemia and supported a prolonged viraemic state. This may reflect an inability of this host species to clear the virus, rendering them unlikely to survive an infection in the wild.

Warthogs were more resistant to the clinical effects of the virus, but nevertheless supported a productive infection and developed of a specific antibody response. This raises the possibility that, if warthogs were to become infected in the wild, they could serve as important reservoir hosts.

We conclude that wildlife Suidae support productive CSF infection and this may be important in controlling future outbreaks on the African continent.

Acknowledgements

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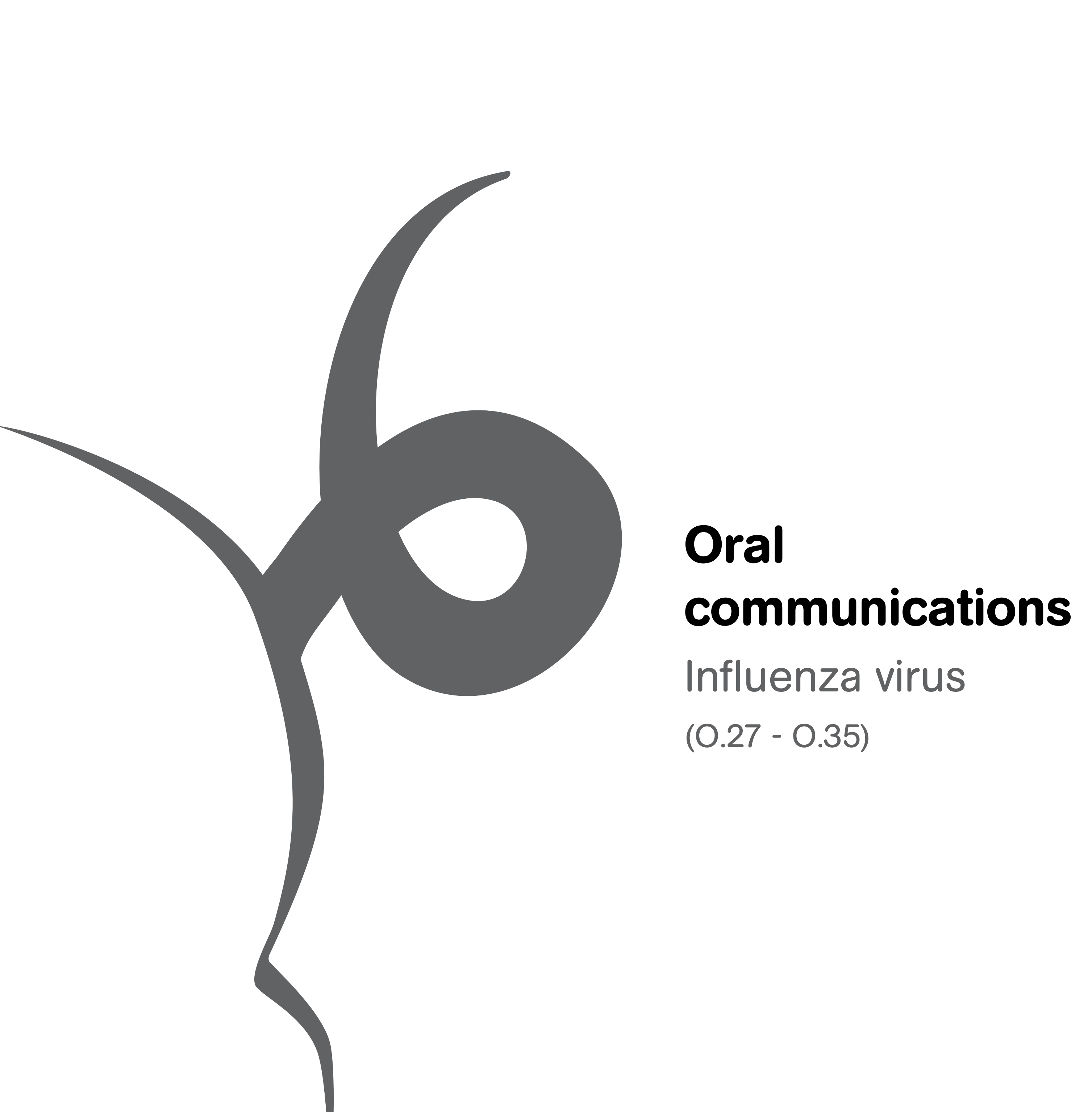

Oral communications

Influenza virus

(O.27 - O.35)

O.27
SWINE INFLUENZA H1N2 REASSORTANT WITH THE PANDEMIC H1N1 (2009) VIRUS IN SOUTH KOREA

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Introduction

Since 2009, the infection of pandemic (H1N1) influenza viruses in human populations has been reported globally, and they were also isolated in Korea swine farms as a result of human-to-swine transmission. In addition, the emergence of novel reassortant H1N2 swine strain derived from pandemic H1N1 influenza in swine has been raised in many countries including Italy. It was widely known that pigs have a role as “mixing vessels” for influenza viruses, and therefore, the generation of novel rearrangement between Korean H1N2 SIVs and pandemic H1N1 influenza viruses may be occurred. This report revealed reassortant events that the novel reassortment H1N2 viruses between pandemic (2009) influenza H1N1 virus and H1N2 were isolated in Korea for the first time.

Materials and methods

A total of 374 samples including lung, tonsil and nasal swabs from pigs were obtained from 273 swine farms of eight provinces in South Korea from September 2009 to September 2010. SIVs were detected by RT-PCR, targeting matrix gene. Among 51 influenza-positive samples from RT-PCR, one H1N1 and nine H1N2 SIVs were isolated in the Madin-Dardy Canine Kidney cells. Amplification of viral hemagglutinin (H1, H3), neuraminidase (N1, N2), polymerase PB1, PB2, PA, nucleoprotein (NP), matrix, non-structural (NS) genes was performed with each primer set to get genetic information comparing with other reference strains, and SIV sequences were confirmed and searched highly similar sequences by the Basic Local Alignment Search Tool (Blast). The nucleotide sequences were aligned using Clustal X (2.0.12) multiple sequence alignment program, and compared with reference viruses to evaluate the relationship using Bioedit software (7.0.5.3). Phylogenetic trees were constructed by the MEGA (4.0) program. The similarity was determined by comparison with sequences of reference strains available in Genbank.

Results

Among 374 swine specimens examined, 110 samples (29.4%) were detected by RT-PCR, targeting matrix gene. Among the inoculated 51 SIV-positive pooling organ samples, we isolated 10 SIVs in MDCK cells. These were one classical H1N1 SIV subtype and nine H1N2 SIV subtypes from different 8 farms in six province of South Korea except for Cheonnam, Gwangwon. Among 9 H1N2 isolates, new reassortant viruses (Sw/Korea/4940/10, Sw/Korea/4941/10) are characterized by 7 genes belonging to pandemic H1N1 influenza over 96% nucleotide identity and NA genes were closely related to H1N2 SIVs of North America swine strain. Sw/Korea/4382/10 is also characterized by 6 internal genes belonging to pandemic H1N1 influenza and HA, NA gene closely related to H1N2 SIVs of North America and Korea swine strain. The other isolates including H1N1 and six H1N2 isolates (except for Sw/Korea/4382/10,Sw/Korea/4940/10,Sw/Korea/494 1/10) were closely related to triple reassortant SIV strains from North America.

Discussion

These results imply that pandemic H1N1 influenza viruses were transmitted to swine population and then, rearranged with gene segments in Korean swine populations. The co-infection of influenza viruses in swine demonstrates that pigs can behave as “mixing vessels” to create reassortant viruses between mammal and animal. As the isolation rate of pandemic H1N1 from pigs increases in many different countries, the possibility of establishment of pandemic H1N1 in Korean swine populations is also considered. Our report suggests the reassortment events between pandemic (2009) influenza H1N1 virus and H1N2 SIV in Korea for the first time. Examinations of H1N2 reassortment viruses for the establishment and the prevalence in Korea are not known yet, and It is the subject of further study. The isolation of novel reassortant H1N2 virus reveals that the frequency of reassortment events may have increased in Korean pig populations and required for surveillance of H1N2 influenza. This new reassortant could not only have zoonotic potential but be pandemic threat to Korean swine industry.

Acknowledgements

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O.28
PANDEMIC INFLUENZA A H1N1V CIRCULATES IN DANISH PIGS

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Introduction

Influenza subtypes H1N1, H3N2 and H1N2 are circulating in pig populations worldwide. In March-April 2009, a novel pandemic Influenza A H1N1 virus (H1N1v) of swine origin emerged in the human population globally. The first case in pigs was reported from Canada in May 2009 and since then most pig-producing countries have reported cases. To distinguish this new influenza subtype from already circulating strains was a challenge for the veterinary diagnostic laboratories.

We report here the validation of a diagnostic strategy for specific diagnosis of the pandemic H1N1v in Danish pigs and results indicating that this virus has become established in the Danish pig population.

Materials and methods

Routinely, detection of swine influenza virus in clinical specimens is performed by real-time reverse transcriptase PCR assays (rRT-PCR) targeting the M and the NP genes. Alignment of the probe and primer sequences to H1N1v gene sequences from GenBank revealed that these assays most likely would recognize the H1N1v virus, thus not discriminating between the circulating strains and the H1N1v subtype. For specific detection of the H1N1v subtype, an rRT-PCR assay targeting the HA gene developed at the Statens Serum Institute for the diagnosis of H1N1v in humans, was validated for use on pig specimens.

Results

In silico analysis showed that the probe and primers of the H1N1v specific assay had 100% identity to published H1N1v strains and 80-95% identity to classical-swine H1N1 which do not circulate in Denmark. In contrast, there was only up to 60-70% match to the subtypes circulating in Denmark (avian-like H1N1, H3N2, and avian-like H1N2), indicating that these subtypes would not be detected by this assay. The negative outcome by this H1N1v specific assay when testing 76 Danish swine influenza virus positive samples confirmed the specificity of this assay for H1N1v. Test of dilution series of cell culture adapted strains revealed a sensitivity of 1-2 TCID50/ml.

All influenza positive samples from swine submitted to the National Veterinary Institute (NVI) in Denmark during 2009 and 2010 have been tested for H1N1v (table 1). H1N1v was not detected in any of the 2009 samples, whereas samples from 9 different herds in 2010 were positive for H1N1v.

The first two positive H1N1v cases were found in January 2010 and the diagnoses were confirmed on nasal swabs from 60 piglets taken 4 days after the initial sampling. Testing was performed with the H1N1v specific rRT-PCR assay and partial sequencing of the HA gene. In two herds diagnosed in June and August 2010, respectively, follow-up nasal swab samples from piglets taken 5 and 3 weeks after the initial diagnosis, respectively, were positive for H1N1v.

Table 1. Svine influenza virus detected in Danish pigs.

	Submissions (% positive)	H1N1 Avian-like	H3N2	H1N2dk Avian-like	Unknown*	H1N1v
2003	122/16 (13%)	8	5	2	0	
2004	95/20 (21%)	8	5	6	1	
2005	141/29 (21%)	16	4	6	4	
2006	146/27 (19%)	8	4	6	9	
2007	117/36 (31%)	11	5	6	11	
2008	293/90 (30%)	18	3	11	58	
2009	299/81 (27%)	4	3	4	68	0
2010	279/96 (34%)	1	0	1	85	9

*Subtyping unsuccessfull or not attempted beyond H1N1v subtyping.

Discussion

Human H1N1v has been shown under experimental conditions to be able to infect swine and transmit among swine (1). Human exposure is the suggested cause of many first-case country reports of H1N1v in swine around the world. The detection of H1N1v positive herds in Denmark throughout 2010, suggests that H1N1v is now being established in the Danish pig population.

Surveillance of influenza in swine in Denmark has relied exclusively on test of samples submitted to NVI with influenza diagnosis requisitioned. Due to lack of a formal surveillance programme in place, we have currently no overview of the number of H1N1v positive swine in Denmark, but the occurrence of cases even beyond the human influenza season suggests an ongoing swine to swine transmission, and is supported by prolonged detection in two follow-up investigations.

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O.29

PANDEMIC INFLUENZA VIRUS OUTBREAK IN NORWAY 2009/10: A CASE-CONTROL STUDY IN SWINE NUCLEUS AND MULTIPLIER HERDS, PRELIMINARY FINDINGS

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Introduction

The first outbreak of infection with pandemic influenza A (H1N1) 2009 virus (H1N1pdm) in a Norwegian swine herd was recorded on 10th of October 2009 (1,2). Until then the Norwegian pig population was considered free of swine influenza (subtypes H1N1 and H3N2) (3). This abstract describes the preliminary results of a retrospective case-control study to investigate human influenza like illness (ILI) as a risk factor for infection of H1N1pdm in Norwegian nucleus and multiplier herds.

Materials and methods

The study population comprised 118 nucleus and multiplier herds. Three herds were excluded on the basis of uncertain infection status at the time of the study. Of the 115 remaining herds, 47 were nucleus herds, and 68 multiplier herds. All herds were tested serologically or by rRT-PCR during the risk period (30th September 2009 until 31st Oct 2010). Information on clinical history of humans and pigs were collected by questionnaire and telephone interview.

We calculated the odds ratios (OR) for each risk factor and used one-sided Fisher's exact test to calculate statistical significance.

Results

Response rate from farmers was 100%. A total of 20 (43%) of the nucleus herds and 28 (41%) of the multiplier herds were classified as positive.

Table 1: Strength of association (OR) and statistical significance of risk factors

Risk Factors	OR	p-value
Farmer or farm worker or relief farm worker with ILI	4.22	<0.01
Farmer or farm worker or relief farm worker, close family or veterinarian with ILI	4.46	<0.01
Only farm worker with ILI	4.53	0.02
Only farmer with ILI	3.26	0.03
Only relief farm worker with ILI	3.20	0.08
Only close family with ILI	2.09	0.09
Only veterinarian with ILI	2.86	0.37

Chronological disease data from relevant humans and pigs were available from 14 of the 48 positive herds. Twelve of these reported ILI in humans followed by contact with pigs before the pigs began to show clinical signs of H1N1pdm infection. Extrapolated to the whole study population this gives a probability of 65-99 % (95% confidence interval) of humans being infected before the pigs

Discussion

The results from this study indicate that ILI in people with pig contact was a significant risk factor for H1N1pdm infection in pigs. During the human influenza season in Norway 2009/2010, nearly all influenza strains that were subtyped were confirmed to be H1N1pdm (4). Furthermore, genome sequencing of the virus from a pig on one farm confirmed it as identical to the virus from a sick farm staff member at the same farm (1). Our findings are supported by other studies that have shown the reverse zoonotic potential of the H1N1pdm (5).

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O.30
SWINE INFLUENZA ACTIVE SURVEILLANCE IN THE UNITED STATES

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Introduction

Influenza virus has become an important pathogen worldwide. In swine, influenza causes a mild respiratory disease and is considered to have little impact on production or health of the pigs. However, swine do play an important role in the ecology of the disease since humans can become infected with influenza viruses originating from swine (1). Presently, the epidemiology of the virus in swine farms is not well understood (2). Therefore, an active surveillance program can provide information on the epidemiology, ecology and evolution of influenza A viruses in swine.

Materials and methods

Thirty-two conveniently selected commercial pig farms in the United States were chosen to participate in this study. Farms were located in swine dense areas in Illinois, Indiana, Iowa and Minnesota. Thirty nasal swabs were collected from growing pigs every month for 12 consecutive months. Swabs were tested for influenza A viral RNA using a RRT-PCR targeting the matrix gene (3). During collection, the age of the pigs, group clinical signs and history of influenza vaccination were recorded. Association between farm characteristics and presence of influenza virus was performed by chi square statistic. A group of pigs was defined as the 30 pigs that were sampled in a given month.

Results

A total of 11,460 nasal swabs have been collected since Jun 2009 to January 2011. From the total number of swabs collected, 9,002 have been tested. Out of those swabs tested, 380 (4.22%) were influenza A virus RRT-PCR positive. Twenty-nine percent of all positive swabs were from pigs between 13 and 15 weeks of age. Influenza was detected in pigs as young as five weeks of age. The average number of positive swabs in positive groups was 6.4 with a minimum of 1 and a maximum of 29. Twenty-six (81%) out of the 32 participating farms have had at least one influenza positive group. Since the beginning of this project, at least one positive swab has been identified in the participating farms every month with the exception of November 2009. Since June 2009, a total of 301 groups of pigs have been monitored. Fifty-nine (19%) have had at least one positive swab. Seventeen (28%) out of those 59 positive groups had clinical signs on the day of the sampling. Additionally, from the 59 positive groups, 32 (54%) groups had a history of influenza vaccination at the sow source farm. No statistical difference was seen when influenza vaccination history and age were compared between influenza positive and negative groups. However, there were statistical differences ($P<0.01$) between farm types (nursery, wean-to-finish, finishing, farrow-to-finish and gilt developer unit) among positive and negative groups.

Thirteen groups of pigs have been confirmed infected with the 2009 pandemic H1N1 strain, eight groups with H3 subtype and twenty-one with H1 subtype viruses. Subtyping is still being conducted on the remaining positive groups.

Discussion

According to the preliminary results of our study, influenza A virus is present in pigs regardless of the farm type, age, month of the year and vaccination status. In our study, even though detection rate is low, the virus is being detected in populations in which there are no clinical signs at the moment of the sampling highlighting the importance of an active surveillance system.

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O.31

GENETIC DIVERSITY AMONG SWINE INFLUENZA A VIRUS ISOLATES FROM HERDS WITH CLINICAL DISEASES IN ARGENTINA BETWEEN 2008 AND 2010

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Introduction

Epidemiological studies on swine influenza virus (SIV) in South America are scarce. Serological surveys carried out in Venezuela, Colombia and Brazil reveal that different influenza virus strains are currently circulating among pig populations.

Currently, influenza A subtypes H1 and H3 circulate in Argentinean pig populations (1). However, despite the positive serological response, neither clinical disease nor SIV isolation had been reported until the outbreak of H1N1 pdm in a swine farm (2). Here, we report the phenotypic and genotypic diversity of influenza A isolates between 2008 and 2010.

Materials and methods

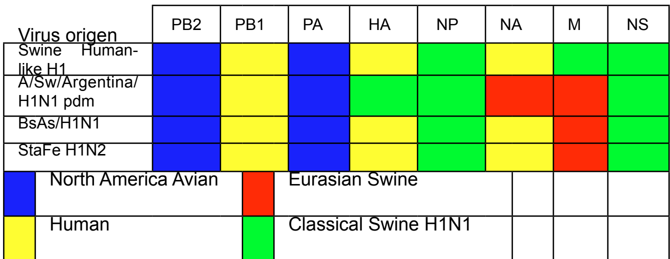
Bronchial swabs and lung samples were obtained from postmortem examined pigs from three herds, two from Buenos Aires Province (herds A and B) and one from Santa Fé Province (herd C).

Outbreaks were reported only in nursery barns. Morbility rates varied between 10 to 40% and mortality from 1.5 to 4% (herd C). The cycle of infection persisted for 2 (herd A) to 8 weeks (herds B and C). Samples were processed with three independent rRT-PCR tests: influenza type A (InfA) directed to the matrix (M) gene, swine influenza (SwInfA) directed to the NP gene and pandemic (H1N1) 2009 virus directed to the HA gene (SwH1). Positive samples by rRT-PCR were processed for virus isolation in MDCK cells. The genome segments of the isolated viruses were amplified by RT-PCR and sequenced directly. Regions corresponding to the entire open reading frame all segments were obtained. Nucleotide BLAST analysis was used to identify the most closely related influenza A virus gene for each respective segment.

Results

H1N1 pdm was identified in samples from farm A. Samples from farm B were positive to influenza A. Further, rRT-PCR demonstrated the H3N2 subtype and the partial sequence of the eight genome segments revealed a high nucleotide identity with wholly human origin influenza A. One year later, 9 samples from the same farm were positive for InfA and SwInfA, but negative for the SwH1 test. Eight samples from farm C were positive for InfA and SwInfA, but only three were positive for the SwH1 test. Samples from farm B and C, InfA/SwInfA-positive but SwH1-negative were grown in tissue culture in MDCK cells, and two different SIV isolates, herein referred as BsAs/H1N1 and StaFe/H1N2, were isolated from farm B and C respectively (see table 1). Molecular analyses indicated that the internal genes were similar to the reassortant internal genes of H1N1pdm viruses. However, the HA and NA genes were of human origin.

Table 1.Lineages of reassortant swine viruses



Virus origen	PB2	PB1	PA	HA	NP	NA	M	NS
Swine Human-like H1	blue	yellow	blue	yellow	green	yellow	green	green
A/Sw/Argentina/H1N1 pdm	blue	yellow	blue	green	green	red	red	green
BsAs/H1N1	blue	yellow	blue	yellow	green	yellow	red	green
StaFe H1N2	blue	yellow	blue	yellow	green	yellow	red	green

Colour	Lineage	Colour	Lineage
blue	North America Avian	red	Eurasian Swine
yellow	Human	green	Classical Swine H1N1

Discussion

Infection of pigs with the wholly human H3N2 subtype can occur under natural conditions in Europe and China (3). In North America, only two wholly human influenza viruses have been identified without sustained circulation (4). However, the isolate H3N2 is transmitted efficiently among pigs. Phylogenetic analysis of BsAs/H1N1 and StaFe/H1N2 confirmed that they were reassortant viruses from H1N1pdm. The above results support the need for a more extensive surveillance of influenza virus in Argentinean pig populations.

Acknowledgements

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O.32

LONGITUDINAL STUDY OF SWINE INFLUENZA VIRUS INFECTION AND PHYLOGENETIC ANALYSIS OF H1N1 ISOLATED IN A FARROW TO FINISH FARM

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Introduction

Swine influenza is assumed to affect farms explosively, producing outbreaks that affect most of animals in few days. However, the description of dynamics of SIV within a pig farm is scarcely known beyond the picture of an epidemic outbreak. The present study was aimed to determine the dynamics of SIV in a batch of a farrow-to-finish farm.

Materials and methods

A farrow-to-finish farm previously known to have SIV seropositive sows was selected for the present study. A whole weaning batch (n=121) of 3-week-old piglets was followed until 24 weeks of age. Nasal swabs were taken weekly between the 3rd and 13th week of age and afterwards, pigs were sampled at 14, 15, 17, 20 and 24 weeks of age. In each visit to the farm a clinical inspection of pigs was performed. Viral shedding was assessed in nasal swabs by means of a TaqMan one-step RRT-PCR using Fast7500 directed to the *M* gene of SIV using primers and a probe described before (1). All RRT-PCR positive samples were inoculated in both embryonated eggs and MDCK cells. Viral isolates were subtyped by RT-PCR (2) and sequenced for the hemagglutinin and neuraminidase genes (H1, H3, N1, N2). Multiple sequence alignment and phylogenetic analysis were done using MEGA 3.1.

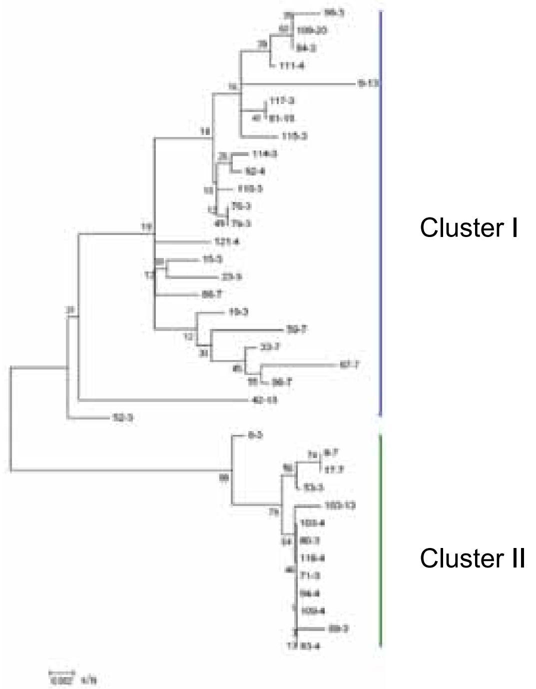
Results

In total 60 animals (49.6%: $IC_{95\%}$: 40.1-58.6%) were positive at least once. Four waves of viral circulation were observed: in suckling piglets (at 3-4 weeks of age), in weaners (at 7 weeks of age), in early fatteners (13 weeks of age), and in late fatteners and finishers (at 15, 17, and 20 weeks of age) with weekly incidences ranging from 2.0 to 28.8%. All isolates belonged to the H1N1 subtype. Based on sequencing results, H1 was similar in all the examined isolates (≥99.4% similarity). In contrast, two different clusters (I and II) could be determined with regards to N1 (Figure 1). Isolates of both clusters were found contemporarily in three of the four viral waves observed in suckling pigs, weaners and fatteners. Actually, two animals got infected twice (different weeks) by the same viral strain and one got infected by the two different H1N1 strains circulating in the farm.

Discussion

Results of the present study show that co-existence of different SIV strains belonging to the same subtype can occur in a pig farm. Moreover, the circulation of these two strains took place in pigs of all ages from 3-weeks onwards. This indicates that in endemic farms, epidemiology of influenza is probably more complex than previously thought. Also, the fact that some animals were infected twice by the same strain indicates that, most probably, the immunity afforded by the first infection was only partial. Moreover, the co-existence of two viral strains in the same batch of animals suggests that the potential for reassortants to arise in a pig farm is high.

Figure 1. Phylogenetic tree (N1) of SIV isolates (the last number indicates the age of animals).



Acknowledgements

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O.33
DYNAMICS OF INFLUENZA TRANSMISSION IN VACCINATED AND NON-VACCINATED PIG POPULATIONS

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Introduction

The ecology of influenza virus is complex and pigs play a central role in it. In North America pig herds are endemically infected and influenza infections are observed year around. Despite its widespread prevalence limited information is available on the factors that contribute to the infection dynamics and transmission. Understanding the factors that contribute to influenza transmission in populations is crucial to prevent influenza spread between animals and from animals to people. The objective of this study was to determine transmission parameters for an H1N1 influenza virus in vaccinated and non-vaccinated populations of pigs and assess the effect of vaccination on influenza transmission.

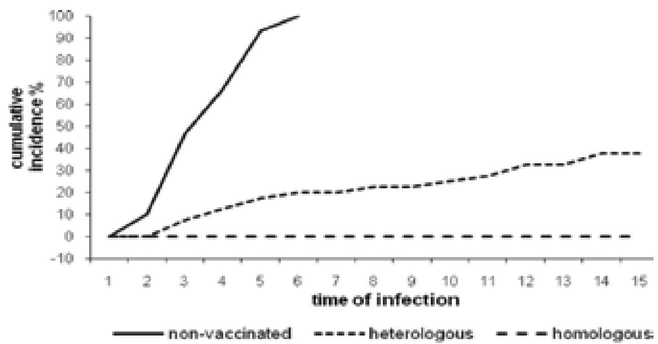
Materials and methods

One hundred influenza free 3-week-old pigs were distributed in replicates of 10 in 3 groups as follows: a) non-vaccinated (NV), b) heterologous (HE) vaccinated and c) homologous (HO) vaccinated. There were 3 replicates for the NV and the HO and 4 for the HE group. Pigs in the HE group were vaccinated with a commercially available swine vaccine containing one H3N2 and two H1N1 strains distinct from the challenge strain. Pigs in the HO group were vaccinated with a vaccine prepared with the challenge strain. The challenge strain was a triple-reassortant H1N1 strain (A/Sw/IA/00239/04 H1N1) recovered from a respiratory field case. In separate rooms, influenza negative pigs ("seeders") were inoculated with the challenge strain intratracheally and intranasally and two days post challenge, the seeder pigs were placed in contact with the pigs in each replicate of the treatment groups. Transmission was evaluated by collecting nasal swabs from all pigs on a daily basis and up to 14 days post contact. Nasal swabs were tested by RT-PCR. Transmission parameters including R (reproductive ratio) were calculated using a generalized linear modeling (GLM) method using a complementary log-log link function as offset variable.

Results

Transmission parameters including the R values differed significantly between vaccinated and non-vaccinated pigs. A significant reduction in transmission was observed in the vaccinated groups where R (95%CI) was 1 (0.53-1.67) and 0 for the HE and the HO group respectively, compared to 10.66 (7.19-15.19) in unvaccinated pigs ($p<0.001$). Transmission in the HE group was reduced, delayed and variable compared to the unvaccinated group. Transmission could not be detected in the homologous group. Figure 1 shows the time course of infection for control and vaccinated groups.

Figure 1. Cumulative number PCR+ pigs overtime.



Discussion

Results from this study indicate that influenza vaccines can be used to decrease influenza transmission but they can also contribute to maintaining endemic infections in populations, especially when vaccine strains differ from the circulating ones. An R >1 indicates that an infectious agent will be able to transmit and spread to other susceptible animals. In this study, the NV had an R significantly higher than 1 while the HE group had an R value around 1 with a confidence interval above and below 1 indicating the variability that populations with partial immunity represent. This is relevant because the results of the present study support field observations where a significant variability in transmission dynamics is observed in vaccinated herds. This is the first study that evaluates transmission rates for influenza in pigs.

Acknowledgments

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O.34

DISTRIBUTION OF INFLUENZA A VIRUSES OF AVIAN AND SWINE ORIGIN AND THEIR SIALIC ACID RECEPTORS IN EXPERIMENTALLY INFLUENZA INFECTED PIGS

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Introduction

Pigs are considered susceptible to influenza A virus infections from different host origins because earlier studies have shown that they have receptors for both avian (sialic acid-α-2,3-terminal saccharides (SA-α-2,3)) and swine/human (sialic acid-α-2,6-terminal saccharides (SA-α-2,6)) influenza viruses in the upper respiratory tract (1). Furthermore, there are reports of experimental and natural infections in pigs with influenza A virus from avian and human sources. Based on this it has been believed that pigs can act as mixing vessel for influenza A viruses to create new viruses.

This study has investigated the receptor distribution in the entire respiratory tract of the pig and predilection sites of respectively swine- and avian influenza virus.

Materials and methods

Experimental infections were performed in different groups of pigs. Swine influenza inoculated pigs: A/swine/Denmark/19126/1993 (H1N1) (n = 2 pigs) and A/swine/Denmark/10074/2004 (H1N2) (n = 2 pigs). Avian influenza inoculated pigs: A/duck/Denmark/65472-26/2003 (H4N6) (n = 4 pigs).
Mock inoculated pigs: pure allantois harvest (n = 4 pigs). Non inoculated pigs (n = 8 pigs).
Day 4 after inoculation pigs were euthanized and tissues from lung (9 sites), trachea, and nose were collected and fixated in 10% formalin.
Immunohistochemistry was performed using antibodies against influenza A virus. Detection of receptors were done using specific lectins *Maackia Amurensis* I, and II (SA-α-2,3), and *Sambucus Nigra* (SA-α-2,6). All receptor stained tissue sections were evaluated separate in respectively influenza affected and non-affected areas.

Results

Swine influenza virus was pronounced in bronchi, but was also present in epithelial cells of nose, and trachea. In severely affected animals epithelial cells of bronchioles and alveolar type I and II epithelial cells were affected as well. Avian influenza, on the other hand, was only found in the lower respiratory tract especially in alveolar type II epithelial cells and sometimes in bronchiolar epithelial cells, but very rarely in epithelial cells of bronchi.

The SA-α-2,6 receptor was by far the most predominant in the pig. All pigs had in all areas of the respiratory tract a pronounced amount of the SA-α-2,6 receptor reaching on average 80-100% lining of the epithelial cells. On the contrary, the SA-α-2,3 was not present (0%) at epithelial cells of nose, trachea, and most bronchi, but was only found in small amounts in bronchioles and alveoli reaching an average of 20-40% at the epithelial cells. Interestingly the receptor expression of both SA-α-2,3 and 2,6 was markedly diminished in influenza affected areas compared to non-affected areas.

Discussion

The study showed that the swine influenza receptor SA-α-2,6 is the predominant receptor in all areas of the respiratory tract of the pig and that the avian influenza receptor SA-α-2,3 mainly is present in alveoli. The results are in agreement with recent studies on influenza receptors in pigs (2, 3). As well as the receptor distribution has resemblance to findings in humans (4). This make the status of the pig as mixing vessel less substantial.

The receptor distribution in this study was in correlation with the predilection sites of respectively swine and avian influenza, where avian influenza infection mainly was found in the alevolar type II epithelial cells. The results are in accordance with human studies showing that it is the lower respiratory tract and alveolar type II epithelial cells avian influenza prefer to infect (5). It could be interesting to investigate the factors that are important for the predilection of alveolar type II epithelial cells of an avian influenza infection in both humans and pigs to further elucidate the pathogenesis.

An interesting finding in the study was that receptors were markedly diminished in areas with influenza infection, and poses questions of what impact this could have on double infection and hence reassortment.

Acknowlegdements

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O.35

COMPARISON OF IMMUNE RESPONSES GENERATED AND PROTECTION TO CHALLENGE IN PIGS VACCINATED WITH CURRENT ADJUVANTED AND NON-ADJUVANTED PANDEMIC H1N1 INFLUENZA VACCINES

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Introduction

In 2009, the World Health Organization (WHO) declared an H1N1 pandemic in response to the emergence and global spread of a novel influenza A virus (H1N1) (1). In the UK, two pandemic influenza vaccines produced from the A/California/07/09 H1N1 v-like strain antigen have been used for the national immunization programme: AS03b-adjuvanted split virion vaccine (Pandemrix, GlaxoSmithKline) or non-adjuvanted whole virion vaccine (Celvapan, Baxter). In the present study, we compared the immune responses generated and protection to challenge in in-bred large white pigs (Babraham line) intramuscularly immunised with these commercial influenza vaccines.

Materials and methods

The vaccination regime in Babraham pigs was similar to that recommended for humans: (i) the adjuvanted split vaccine was administered as a single dose (0.5ml), (ii) the non-adjuvanted whole vaccine was administered twice (two doses three weeks apart), or (iii) the control mock vaccine (AS03b-adjuvanted uninfected allantoic fluid) was administered as a single dose. At various time-points post-vaccination (pv), we assessed the humoral and cellular immune responses generated in individual pigs by various means: cell proliferation of specific $CD4^+$ and/or $CD8^+$ T cell sub-populations by flow cytometry (CFSE), interferon γ (IFNγ) production in cell supernatants by ELISA and IFNγ-producing cell quantification by ELISPOT upon re-stimulation of PBMC *in vitro* with chemically-inactivated A/California/07/09 or UV-inactivated A/England/195/09 H1N1, and antibody (Ab) titres in the serum by ELISA and HI assay.

Three months after the initial vaccination, all pigs were intra-nasally challenged with 10^6pfu of infectious A/England/195/09 H1N1 (human pandemic isolate). Viral RNA levels in nasal swabs from various time points post-challenge were determined by a modified influenza A matrix (M) gene RRT-PCR assay (2).

Results

In response to an *in vitro* stimulation of PBMC with inactivated H1N1 virus, we detected IFNγ-producing cells (cellular fraction) and IFNγ production (cell supernatants) only at day 7pv, and only in pigs vaccinated with the adjuvanted split vaccine. We then assessed the *in vitro* proliferative response to inactivated A/England/195/09 H1N1 in all pigs at day 28pv. Our results demonstrated an influenza-specific recall response in $CD8^+$ (CTL) and $CD4^+CD8^+$ (T_h) cells of pigs vaccinated with the adjuvanted split vaccine. In contrast, only a low level of recall response to A/England/195/09 H1N1 was detected in $CD4^+CD8^+$ (T_h) cells of pigs vaccinated with the non-adjuvanted whole vaccine.

We found influenza-specific Ab response in serum of vaccinated pigs to be dominated by the IgG1 isotype. Pigs vaccinated with the adjuvanted split vaccine exhibited a stronger Ab response after one vaccination as compared to pigs vaccinated with the non-adjuvanted whole vaccine. However, Ab titres in the latter pigs were boosted after the second vaccination, reaching finally higher titres. Similar findings were obtained when considering HI titres.

No, or very mild, clinical signs were observed in all pigs following the infectious challenge. Importantly, the pigs displayed a pattern of nasal shedding that was similar across all three groups, irrespective of the vaccination regime.

Discussion

Our results suggest the non-adjuvanted whole vaccine only generated a weak T_h response, whereas the adjuvanted split vaccine elicited a stronger T_h response associated with a strong CTL response (presumably through cross-priming). Despite the fact that both vaccines also elicited a strong humoral response, none of the pigs were protected from a subsequent challenge as viral RNA continued to be shed from the nasal cavity. A similar study was conducted in ferrets by the pandemic influenza vaccine evaluation consortium (PIVEC) (3). This study showed the pandemic H1N1 vaccines slightly limited or had no effect on the viral shedding from the upper respiratory tract, although one of the vaccines did prevent viral replication in the lower respiratory tract (LRT). These results are consistent with our findings, although viral replication in the LRT of pigs was not assessed.

In conclusion, data provide support for the use of the pig as a valid experimental model for influenza infections in humans, including the assessment of protective efficacy of therapeutic interventions.

Acknowledgements

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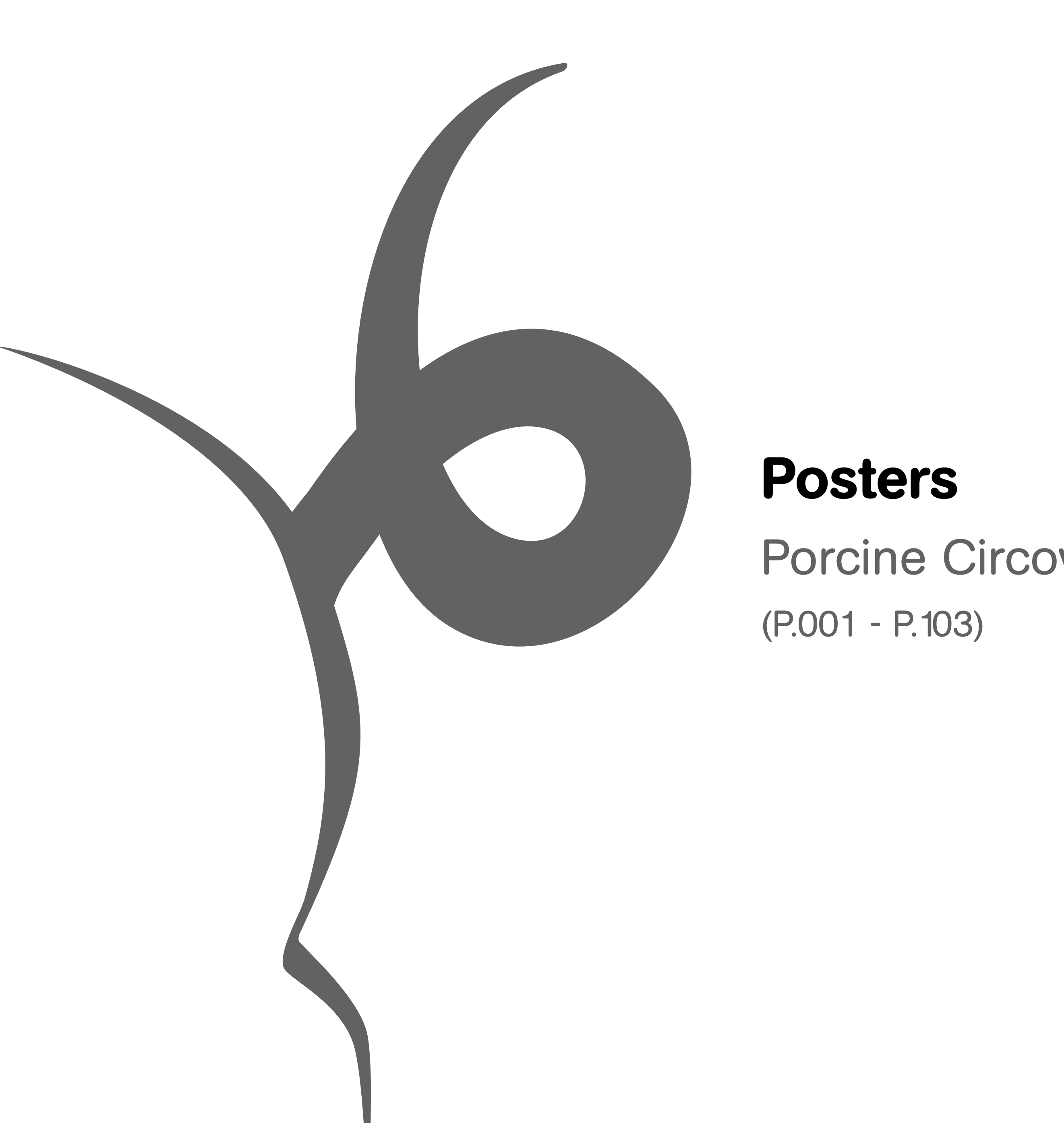

Posters

Porcine Circovirus

(P.001 - P.103)

P.001

ORF3 OF THE PORCINE CIRCOVIRUS 2 ENHANCES THE IN VITRO AND IN VIVO SPREAD OF THE VIRUS

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Introduction

The ORF3 protein of the pathogenic porcine circovirus 2 (PCV2) causes apoptosis of the virus-infected cells. In PCV2-infected piglets, ORF3 induces B and CD4 T lymphocyte depletion and lymphoid organ destruction and the ORF3-deficient PCV2 is attenuated in its pathogenicity. In addition to its role in causing the apoptosis of the immune cells, characteristic of the PCV2 infection associated disease conditions, the ORF3 also plays a role in the systemic dissemination of the PCV2 infection.

Materials and methods

The spread of the PCV2 *in vitro* and *in vivo* were measured by quantifying the viral genome copy numbers using real-time quantitative PCR. The kinetics of wildtype PCV2 spread, both *in vitro* and *in vivo*, were compared with the spread of (a) the ORF3 mutant PCV2 and (b) the wildtype PCV2, in the presence of Z-VAD fmk (a potent caspase inhibitor).

Results

Our observations showed that the kinetics of the spread of PCV2, both *in vitro* and *in vivo*, was increased in the presence of ORF3 expression. The presence of the Z-VAD fmk, which abolished the apoptotic activity of ORF3, caused a reduction in the kinetics of the spread of the wildtype virus. Further, the apoptosis induced by ORF3 causes a greater accumulation of the PCV2 genome in the macrophages.

Discussion

Our experiments showed that ORF3 expedites the spread of the virus by inducing the early release of the virus from the infected cells. Further, in PCV2-infected mice, the ORF3-induced apoptosis also aids in recruiting macrophages to phagocytize the infected apoptotic cells leading to the systemic dissemination of the infection. The apoptotic activity of the ORF3 of PCV2 hence lends advantage to the spread of the virus.

Acknowledgements

This work was supported by a grant from the Temasek Lifesciences Laboratory, Singapore.

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P.002

THE ORF3 PROTEIN OF PORCINE CIRCOVIRUS TYPE 2 IS INVOLVED WITH CELLULAR PATHOLOGY DURING VIRAL INFECTION

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Introduction

The ORF3 protein of porcine circovirus type 2 (PCV2) causes apoptosis in virus-infected cells and is not essential for virus replication. The ORF3 protein plays an important role in the pathogenesis of the PCV2 infection in mouse models and SPF piglets. Our previous studies using yeast two hybrid system, have shown that the ORF3 protein interacts with the porcine homologue of Pirh2 (pPirh2), a p53-induced ubiquitin–protein E3 ligase, which regulates p53 ubiquitination. Here, we present our study analyzing the details of the molecular interaction between these three factors.

Materials and methods

In vitro pull down assays were performed using the three proteins to study the nature of interaction between them. Next, the interacting domains and residues of the ORF3 protein and the pPirh2 were mapped using co-immunoprecipitation experiments and pull down assays. Subsequently, ORF3 mutants which do not interct with the Pirh2 were generated. The effect of ORF3 and the non-interacting ORF3 mutant on the pPirh2 were studied in PK-15 cells. The localization and the stability of the pPirh2 were studied using immuno fluorescence assay and cycloheximide treatment assay, respectively.

Results

Our experiments, in vitro and in vivo, show that ORF3 protein competes with p53 in binding to pPirh2. The amino acid residues 20 to 65 of the ORF3 protein are essential in this competitive interaction of ORF3 protein with pPirh2 over p53. The interaction of ORF3 protein with pPirh2 also leads to an alteration in the physiological cellular localization of pPirh2 from nucleus to cytoplasm and a significant reduction in the stability of pPirh2. In the presence of ORF3, the decrease in the Pirh2 level correlates to the increase in p53 levels.

Discussion

The ORF3 expressed during the PCV2 infection binds with the cellular pPirh2 and prevents its translocation to the nucleus. Further, the ORF3 competitively blocks the interaction between pPirh2 and p53. These events prevent the ubiquitination of p53 by pPirh2 and lead to the deregulation of p53. The ORF3 thus causes the increased p53 levels and apoptosis of the infected cells.

Acknowledgements

This work was supported by a grant from the Temasek Lifesciences Laboratory, Singapore.

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P.003

CYTOTOXICITY OF ORF3 PROTEINS FROM A NONPATHOGENIC AND A PATHOGENIC PORCINE CIRCOVIRUS

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Introduction

Porcine circoviruses (PCV) type 1 and 2 are small, non enveloped viruses with single-stranded circular DNA (1). PCV2 has been recognised as the causative agent of PCV-associated diseases (PCVAD), whereas no pathogenic effects are ascribed to PCV1. Analysis of PCV1 and PCV2 variants shows a consistent single-nucleotide substitution in the coding sequence of open reading frame *(*ORF)-3 of PCV2 resulting in a protein half the size of PCV1-ORF3 with only 60% amino acid sequence identity (2). PCV2-ORF3 has been characterized as an inducer of apoptosis (3). The abrogation of ORF3 expression attenuated PCV2 pathogenesis both in mice (3) and specific-pathogen-free piglets (4), suggesting that ORF3 is involved in PCV2 pathogenesis. We hypothesise that if ORF3 is a determinant of virulence of PCV2 via its apoptotic capability, PCV1-ORF3 would lack the ability to induce apoptotic cell death. Our main objective is to determine the differences in the proapoptotic properties between PCV1 and PCV2 ORF3.

Materials and methods

Different PCV1 and PCV2 ORF3, expressed as fusion proteins to eGFP have been used to transfect human (293Tand A549) and pig (PK15) epithelial cell lines, as well as primary kidney epithelial cells (PPKC). These constructs were characterized with regard to their ability to cause cellular morphological changes, detachment, death and apoptosis via biochemical markers, such Annexine V (using flow cytometry), PARP cleavage and caspase expression (using western bloting).

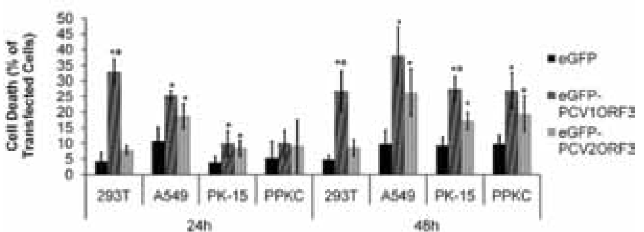
Results

PCV1-ORF3 induced significantly more apoptotic cell death than PCV2-ORF3 and was capable of killing cells of human and porcine origin whereas PCV2-ORF3 only killed porcine cells (figure1). PCV1-ORF3 induced cell death by a caspase-dependent mechanism and also induced PARP cleavage in a caspase-independent manner. Truncation of PCV1-ORF3 and elongation of PCV2-ORF3 proteins revealed that the first 104 amino acids contain a domain capable of inducing cell death, while the C-terminus of PCV1-ORF3 contains a domain possibly responsible for enhancing cell death.

Discussion

Our findings reveal that PCV1-ORF3 is a potent inducer of apoptotic cell death and is more cytotoxic than PCV2-ORF3 under the given experimental conditions. The fact that PCV1 does not appear to be cytopathogenic to infected cells or infected pigs could suggest that this protein is not expressed in the course of infection or that other virus or host proteins counteract its action. Alternatively, during the course of PCV1 infection, PCV1-ORF3 may be expressed only in those cells that are refractive to PCV1-ORF3-mediated apoptosis. The latter hypothesis is supported by our results demonstrating that different cell types exhibited various levels of susceptibility to ORF3-induced cell death. The ability to induce apoptosis to a greater extent in infected cells may actually be more detrimental to the virus, as it allows the immune cells to recognize and clear infected cells without inducing inflammatory responses.

Figure 1: Cytotoxic effect of PCV1-ORF3 and PCV2-ORF3 on cultured cells.



Acknowledgements

This work was supported by NSERC, ALMA, the University of Calgary Faculties of Medicine and Veterinary Medicine.

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P.004
INVOLVEMENT OF RGS16 IN PCV2 PATHOGENESIS

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Introduction

The regulator of G protein signaling 16 (RGS16) is a target for a porcine 2 circovirus type-2 protein (PCV2) and appears to participate in the translocation of the viral ORF3 protein into the cell nucleus, suggesting that the observed interaction may play an important role in the infection biology of porcine circovirus (1). The role of RGS16 in viral pathogenesis has never been reported before, suggesting that it may be involved in new antiviral/virus induced pathways. Consequently, the putative interaction between a PCV2-ORF3 encoded protein and a porcine homologue to RGS16 deserves further studies.

Materials and methods

Infecting C57BL/6 RGS16-/- and wt mice with PCV2. The RGS16 knockout mice generated on C57BL/6 genetic background were obtained as a generous gift from Prof Kirk Druey, NIAID, Bethesda, USA. PCV2 virus was isolated on PK15A cells in the Uppsala University Biomedical Center. Nine weeks old mice (4 individuals per group, all males) were infected by intraperitoneal injection of 400 pfu of PCV2.

Sample preparation from virus infected mice. Peritoneal macrophages and samples from spleen and thymus were taken on 43th day post infection. Cells from 1/4 of the spleen and thymus were fixed in 4 % paraformaldehyde and immunostained with antibodies specific to RGS16 and PCV2 ORF3. The rest of the cells were used for LPS activation experiments to analyze their gene expression profile.

Indirect immunofluorescence labeling. The endogenous expression of PCV2 coat protein in mouse spleen, brain and thymus post intraperitoneal infection was determined by indirect immunofluorescence (IF) labeling using a porcine polyclonal antibody to PCV2 (kindly provided by Prof. Caroline Fossum) and a secondary PE conjugated rabbit serum to porcine immunoglobulins.

Results

1. The immunoregulatory functions of RGS16 are affected by PCV2 infection. To confirm the impact of RGS16 *in vivo*, RGS16 knockout mice in comparison with wt C57BL/6 of the same age was used. It has been shown that mice could get naturally infected by PCV2 in contaminated farms, and may even constitute an effective transmission vector for the virus (2,3). One of the clinical signs of the PCV2 infection in pig is that lungs do not collapse when the thoracic cavity of the animal is opened (4,5). The same phenomenon in infected mice (both RGS16-/- and wt) could also be seen in this study, further confirming that mice have effectively developed a PCV2 infection.

2. RGS16 negatively controls CD14 and C5aR in PCV2-infected mice. The impact of RGS16 on the expression of activation markers CD14 and C5aR was analyzed using qPCR in LPS-activated and control peritoneal macrophages from either RGS16 KO or wt PCV2 infected mice. The expression levels of both markers appeared to be clearly upregulated in RGS16 KO mice when compared to wt mice. This observation shows that the expression of these markers is efficiently repressed by RGS16, even when cells are incubated with LPS.

Discussion

We used RGS16 KO mice to clarify the impact of RGS16 on the activation of myeloid cells. The results presented here imply a positive correlation between RGS16 and the production of CD14, C5aR. The results obtained indicate upregulation of CD14, a key membrane binding site for LPS as part of TLR4 complex, in response to monocyte activation, a result that has also been reported before (6,7). However, C5aR, the receptor for complement component C5a, was also upregulated, which contrasts with the results of earlier studies (8).

Acknowledgements

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P.005
IDENTIFICATION OF MICRORNAs IN PCV2 INFECTED AND NON-INFECTED PIGS

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Introduction

MicroRNAs (miRNAs) are a new class of small non-coding RNAs that regulate gene expression post-transcriptionally. miRNAs play an increasing role in many biological processes (1). The study of miRNA-mediated host-pathogen interactions has emerged in the last decade due to the important role that miRNAs play in the antiviral defence (2).

Porcine circovirus type 2 (PCV2) is the essential aetiological infectious agent of postweaning multisystemic wasting syndrome (PMWS) and has been associated with other swine diseases (3).

The objective of this study was to characterize miRNAs produced in tonsil and mediastinal lymph node of experimentally infected pigs with PCV2.

Materials and methods

Four pigs were intranasally inoculated with $7x10^{4.8}TCID_{50}$ of PCV2 isolate Sp-10-7-54-13 (4) and 2 control pigs received PBS by the same route. At 21 days post-inoculation (dpi), animals were euthanized. Tissue samples were (i) collected in formalin for pathological studies (histopathology and PCV2 detection by *in situ* hybridization (ISH)) and (ii) lysed in Trizol (Invitrogen) for total DNA and RNA isolation (PCV2 load quantification and small RNA libraries construction). A real time quantitative PCR (5) was performed on selected tissues to quantify PCV2 load.

RNA integrity and quantity was assessed with the 2100 Bioanalyzer (Agilent). Enrichment of small RNA fraction was done by excising the 15-25 nt size by PAGE. A miRNA library was constructed in a two-step ligation procedure with the 3' and 5' adaptors from IDT technologies. Amplification by RT-PCR was carried out with fusion primers containing sequences complementary to the 3' and 5' adaptors used for miRNA library construction and sequences complementary to the A and B adaptors used for high-throughput (HT) sequencing with the GS FLX 454 (Roche). Multiplex identifiers, including a five-nucleotide code, were used in fusion primers to allow distinguishing among the 12 samples. PCR products were cloned and sequenced as a quality control step of miRNA library construction. Validated amplicons were analyzed by HT sequencing.

Results

No histopathological lesions were observed in any sample analyzed. Also, none of them were positive by ISH. PCV2 loads in tissues are summarised in table 1. Finally, tonsil and mediastinal lymph node were selected to prepare small RNA libraries. Orthologous miRNAs not previously described in swine as mir93 and mir200c have been identified. Several candidate miRNAs that can be produced in response to infection with PCV2 have been identified. Also, the possibility that PCV2 encode miRNAs was explored.

Table 1. Genome copies/mg detected by qPCR in tissues of infected (No. 3 to 6) and non infected (No. 1 and 2) pigs with PCV2.

	Animal No.					
Tissue	1	2	3	4	5	6
Spleen	-	-	$3.2x10^6$	$2.9x10^5$	$7.2x10^4$	$4x10^5$
Inguinal ln	-	-	$1.2x10^7$	$3.3x10^5$	$1.7x10^6$	$8.9x10^5$
Kidney	-	-	-	-	-	-
Tonsil	-	-	$5.2x10^4$	$1.8x10^6$	$2.1x10^6$	$8x10^5$
Thymus	-	-	-	-	-	-
Mediastinal ln	-	-	$8.2x10^6$	$1.9x10^7$	$3.8x10^7$	$4.6x10^7$
Lung	-	-	$6x10^4$	$2.1x10^5$	$1.1x10^5$	$9.2x10^4$
Mesenteric ln	-	-	$2.5x10^5$	$7.6x10^5$	$7.2x10^4$	$1.1x10^6$

Discussion

To the authors' knowledge, this is the first study on miRNA gene expression in pigs infected with PCV2 using a deep sequencing approach. Besides, because PCV2 is not included in the category of RNA viruses and cytoplasmic DNA viruses which appear not to encode miRNAs (6), it was a candidate to explore if expresses miRNAs. The role of miRNAs in the regulation of the mechanism involved in the virus-host interaction must be helpful in the understanding of the viral infection and the development of the disease.

Acknowledgements

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P.006

PHYLOGENETIC ANALYSIS OF PORCINE CIRCOVIRUS TYPE 2 (PCV-2) IN FETUSES AND STILLBIRTH WITH NATURAL INFECTION

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Introduction

Porcine Circovirus Type 2 (PCV2), the infectious agent of PCVAD (PCV2 associated diseases) is also implicated in reproductive failures in swine (1,2). Vertical transmission seems to be an important route of infection by PCV2, which has been detected by nested-PCR in organs of fetuses naturally infected (1,3).

The PCV2 isolates can be classified in PCV-2a, PCV-2b and PCV-2c genotypes based in its genome diversity. The genotype 2b has been more commonly associated with outbreaks of PMWS in North America and some European countries (4).

This study aims to perform a genetic analysis of the genome of PCV-2 viruses identified by PCR in aborted or stillborn fetus from a preview study (2).

Materials and methods

Samples from field cases, as aborted fetuses, mummified, stillborn and fragile piglets were collected and processed in order to detect PCV2 DNA and antigens. Samples were collected from 21 farms previously identified with occurrence of reproductive losses, and a total of 169 fetuses were necropsied. Fragments of organs, including heart, lungs, liver, kidney, lymphoid organs and nervous tissues were processed for viral and histopathological diagnostic. For viral isolation, DNA was extracted and nested-PCR reactions were performed using specific primers to detect sequences of PCV2 (5). Sequencing from the positive tissues was performed as previously described method and primers (6). The sequencing reactions employed BigDye Terminator chemistry and the products were run on an Applied Biosystems 3130xl Genetic analyzer. Consensus sequence was generated using the SeqScape v2.5 software (Applied Biosystems). A phylogenetic tree from partial genome sequencing of PCV-2 was constructed using the neighbor-joining method, using Kimura 2-parameter model, with the 500 reps of bootstrap in the MEGA 5.01 software based on nucleotide sequences.

Results

From 169 fetus examined by PCR, 29 were positive (17.1%). Virus sequences were found in several tested organs. From the positive ones, it was possible to perform partial sequencing of DNA from 5 fetuses. The sequence analyses determined that these viruses have a similar sequence and they were classified in PCV2a genotype as it is shown in Figure 1.

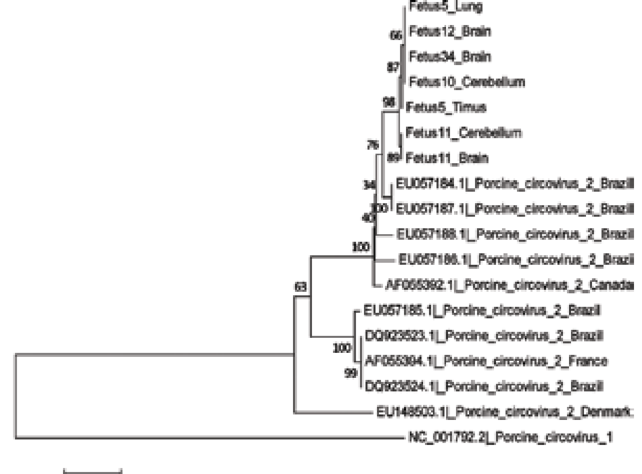

Figure 1: Phylogenetic tree, all 7 samples from this study were classified as PCV-2a genotype together with other Brazilian PCV-2a isolates from a previous work. Other Brazilian isolates were grouped with PCV-2b. PCV-1 genome was used as an out group.

Discussion

The results of this work are corroborating with the reports of association between PCV-2 and reproductive failure (1, 3). In a preview study it was shown the genetic diversity of Brazilian isolates (7), which also can be seen here in the Figure 1. The analysis of the partial sequenced genome reveled that all 7 samples of this work are grouped together with PCV-2a (4). In contrast, European isolates from reproductive failures include also PCV-2b (8). circumstantial Herein, the findings might be related to the strains which are been successfully transmitted in Brazilian swine herds.

Also, the sequencing of the whole genome of these samples – which is underway - will help to make a definitive classification of these viruses as PCV-2a.

Acknowledgements

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P.007

PHYLOGENETIC ANALYSIS OF PORCINE CIRCOVIRUS TYPE 2 (PCV2) ISOLATES IN CROATIA - UPDATE

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Introduction

Porcine circovirus type 2 (PCV2) is non-enveloped virus (17 nm), contains a circular, single-stranded DNA genome, and it belongs to the genus Circovirus of the family *Circoviridae*. Currently, three different phylogenetic subtypes of PCV2 are recognized (PCV2a, PCV2b and PCV2c (1). Clinical PCV2 infection is mainly associated with postweaning multisystemic wasting syndrome (PMWS), but there is an evidence that PCV2 is involved in other so called porcine circovirus type 2 associated diseases (PCVDs or PCVADs). PMWS in Croatia was for the first time recognized during health status monitoring in 2001 in a large pig production unit (2) and later it was also described in wild boars (3). The aim of this study was to sequence and perform a phylogenetic analysis of PCV2 isolates from positive samples from different pathological conditions identified as PCVDs and to compare the newly described genomes with previously published data and sequences deposit in GenBank.

Materials and methods

Altogether 85 samples of PCV2 containing organs were collected and processed for PCR: 63 samples from reproductive disorders, 14 samples from pigs affected with interstitial nephritis and nephrosis, 3 samples from wild boar PMWS cases and 5 samples from proliferative and necrotizing pneumonia. After DNA extraction, PCR was performed with 3 pairs of primers. PCR products were purified and sequenced to determine a complete genome.

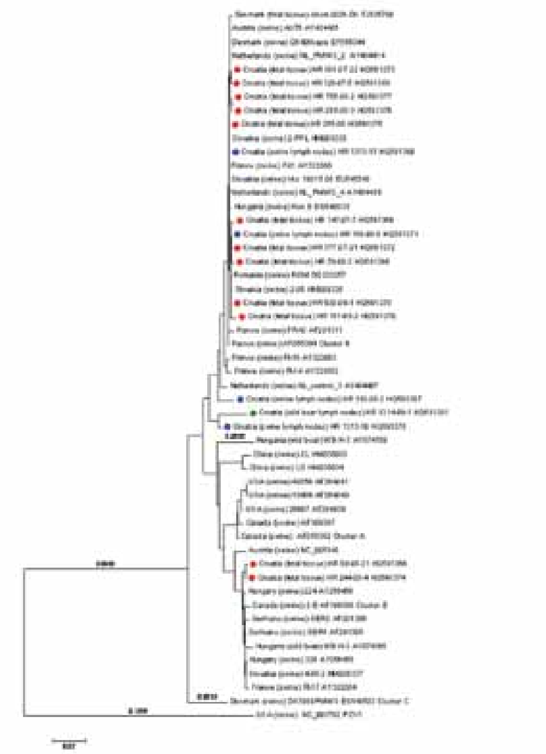
Results

A 17 new complete genome PCV2 sequences were determined (Fig 1).

Discussion

The results of this study indicated that a high homology exists among Croatian PCV2 genomes. Most of them belonged to subtype B and were highly homologous. However, PCV2 subtype A was also present.

Fig 1. Neighbour-joining analysis of complete genome PCV2 sequences showing the phylogenetic relationship between Croatian PCV2 isolates and other related isolates from the GenBank. (Red dots are PCV2 of fetal origin, blue dots are from pigs and green dot is from wild boar).



Acknowledgements

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P.008

GENETIC CHARACTERIRIZATION OF PORCINE CIRCOVIRUS TYPE 2 (PCV2) STRAINS ISOLATED FROM PORCINE CIRCOVIRUS ASSOCIATED DISEASES (PCVAD) PIGS IN THAILAND

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Introduction

Porcine circovirus type 2 (PCV2) was first recognized as a primary causative agent of post weaning multisystemic syndrome (PMWS) in Canada in 1991(1). Consequently, it has been reported in almost pig production countries and currently, all associated diseases linked to PCV2 are included under the term porcine circovirus associated diseases (PCVAD). PCV2 could be divided into 2 major genotypes referred to Genotypes 1 and 2 (2). In Thailand, PMWS was firstly reported in 1998. However, only one isolate of Thai PCV2 from our group has been submitted to GenBank (THA-01NP1, AY864814).

Therefore, the objective of this study was to determine the genetic characterizations of PCV2 isolates from PCVAD pigs in Thailand.

Materials and methods

Samples from necropsy pigs submitted to Chulalongkorn University - Veterinary Diagnostic Laboratory (CU-VDL) and Department of Pathology were included in this study. These samples were kept in -80°C until tests. A full-length ORF2 *gene* of PCV2 was amplified by PCR with forward primer, PCV2-f1 (5’-CCA TGC CCT GAA TTT CCA TA-3’) and reverse primer PCV2-r1 (5’-ACA GCG CAC TTC TTT CGT TT-3’) (3). All 12 Thai PCV2 sequences were analyzed together with 19 representative ORF2 sequences reported in GenBank. A phylogenetic tree was constructed by MEGA 4 software using the neighbor-joining method with 1000 bootstrapping replicates (fig.1).

Results

All 12 Thai ORF2 of PCV2 sequences in this study revealed nucleotide identities ranged between 97.7 - 99.8% (data not shown). The phylogenetic analysis of the 12 Thai PCV2 isolates revealed that all studied Thai PCV2 sequences belonged to genotype 1 consisting of subgroup 1A/ B (10/12, 83.33%) and 1C (2/12, 16.67%) according to the cluster classification described previously (4).

Discussion

In this study, we characterized and reconstructed the phylogenetic analysis of 12 Thai ORF2 of PCV2 sequences collected from pigs with PCVAD during 2007 - 2010 to accurately determine the cluster relationships. All 12 Thai ORF 2 sequences in this study were closely related. A few specific substitution patterns were observed in amino acid positions (data not shown) and genotype 1, subgroup 1A/B was predominated in Thai pigs with PCVAD in this study.

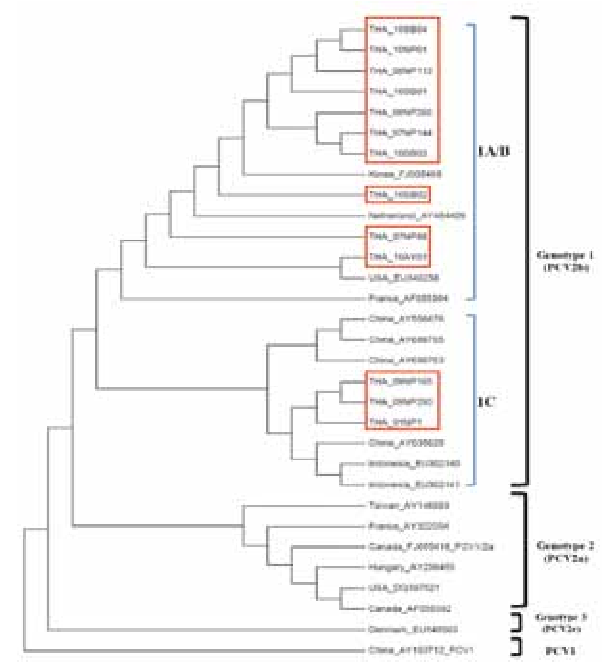

Figure 1: Phylogenetic tree based on neighbor-joining (NJ) method was constructed from the 12 Thai PCV2 sequences in this study with 19 ORF2 of PCV2 sequences reported in GenBank.

Acknowledgements

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P.009

GENETIC CHARACTERIZATION OF PORCINE CIRCOVIRUS TYPE 2 (PCV2) ISOLATED IN PIGS FROM COLOMBIA

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Introduction

Porcine circoviruses (PCV), members of the family Circoviridae,are composed of non-enveloped viruses with a single-stranded circular DNA. Two genetically and antigenically different viruses, PCV1 and PCV2, can be distinguished. While PCV1 is considered nonpathogenic, PCV2 is associated with post-weaning multisystemic wasting syndrome (PMWS) (1).

In Colombia, clinical PMWS associated with PCV2 infection was first reported in 2006 (2), but there are no virus isolates or studies showing PCV2 virus circulating on the field until now. The main goal of this study was to characterize the PCV2 virus present in Colombian swine herds.

Materials and methods

In order to achieve this goal tissue samples from animals showing clinical signs (lymph nodes, lung, spleen, and kidney) were collected from 13 farms with historical records of PMWS during 2006 – 2009.

Diagnosis of PMWS was confirmed for each case (3). Serum samples from five healthy pigs per farm collected between January and December 2009 were also included. DNA extraction was conducted using the DNA Mini kit (Qiagen, USA). The capsid gene was amplified and sequenced (Macrogen, USA) from nucleotides 825 to 1760 using specific primers Fw5´ CCGTTGGAATGGTACTCCTC 3´and Rw 5´ACAGCGCACTTCTTTCGTTT3´.

Strain-specific sequence chromatograms generated using BioEdit package 7.0.5. Resulting in complete ORF2 sequences that were aligned in ClustalW and phylogenetic trees were generated in MEGA4.

Results

PCV2 DNA was detected in 19 samples from different origin. Sequence analysis showed 92%-100% homology between the Colombian PCV2 viruses.

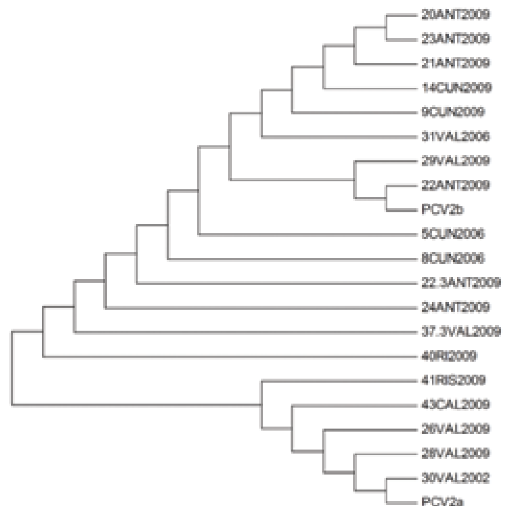
Resulting sequences can be divided into two main clusters supported by high confidence values that matched the two genotypes defined previously; PCV2b (n=14) and PCV2a (n=5). Interestingly, PCV2a and PCV2b sequences were found in healthy as well as in sick pigs. The sequences have relation with Canadian, European and Asian viruses.

Discussion

According to the results, based on the capsid protein encoded by ORF2 gene, two genotypes of PCV2 are present in Colombian pigs showing clinical and temporal differences in its presentation. It is important to notice that PCV2 is circulating in Colombian pigs since 2002 and there is a dynamic in the presence of virus genotypes showed by the analysis of fourteen samples from 2006 -2009 corresponding to PCV2b and four to PCV2a.

According with previous studies (4), PCV2b was associated with a higher prevalence of PMWS outbreaks in Colombian swine herds than PCV2a and the detection of PCV2b genotype since 2006 might explain the PMWS epizootic occurrence in farms of several states of Colombia

Figure 1. Phylogenetic tree of 19 Colombian samples based on ORF2 sequence.



Acknowledgements

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P.010

INCREASED REPLICATION OF PORCINE CIRCOVIRUS TYPE 2 IN PORCINE CELL CULTURES BY STATINS

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Introduction

Porcine circovirus type 2 (PCV2) is a causal agent of postweaning multisystemic wasting syndrome (PMWS) in weaned pigs (1). It is generally accepted that a high PCV2 replication level is a key factor in the induction of PMWS, reviewed by Misinzo et al. (2), but the factors that cause high replication levels of PCV2 in swine remain to be determined. PCV2 replication was enhanced (20 fold increase) in PK-15, SK and ST cells by methyl-β-cyclodextrin treatment (2). In human, statins are used to reduce cholesterol production, but neither the effect of statins on the cholesterol production in swine cells, nor the effect of statins on the level of PCV2 replication in swine cells has been investigated. The aim of this study was to determine reduction of cholesterol production with statins could enhance PCV2 replication in vitro. Enhancement of PCV2 replication in vitro might create a tool to reproduce PMWS in vivo.

Materials and Methods

Lovastatin (Mevinolin, Sigma-Aldrich) and Atorvastatin calcium (Lipitor, Pfizer Pharmaceuticals) were used. To determine the effect of statins on cholesterol depletion, semi-confluent monolayer of PCV- negative PK-15 and primary porcine kidney cells (PPK) were treated with or without atorvastatin (2μM) and lovastatin (2μM) for 0h, 12h, 24h, 36h and 48h at 37°C. The cells were fixed in ice-cold 4% (w/v) paraformaldehyde and cellular cholesterol was visualized by using the fluorescent fungal cholesterol-binding metabolite filipin, as described previously (2).

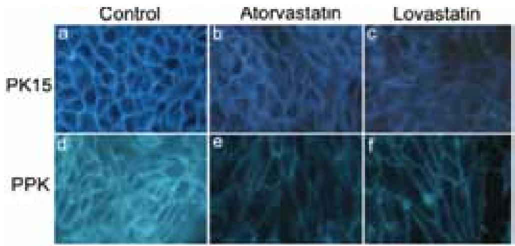
PCV2 strain Stoon-1010 (3) with a dose of $10^{4.3}$ $TCID_{50}$ ml^{-1} was used to inoculate cells for 1 hour at 37°C. To know the effect of statins on PCV2 replication, semi-confluent monolayers of PK-15 and PPK cells were treated with or without atorvastatin (0μM, 0.25μM, 0.5μM, 1μM and 2μM) and lovastatin (0μM, 0.25μM, 0.5μM and 1μM) for 48 hours before and 48 hours after PCV2 inoculation. Cells were fixed in methanol and indirect immunofluorescence staining for PCV2 was performed as described previously (4). The total number of PCV2-positive cells was determined by fluorescence microscopy. These experiments were repeated four times.

Results

As shown in Fig. 1, plasma membrane cholesterol was depleted from statin-treated cells as compared to mock-treated cells. In both PK-15 and PPK cells, Cholesterol depletion was detected starting from 36 hours post-atorvastatin/lovastatin treatment, although in PPK cells it started at 24 hours post-lovastatin treatment (data not shown).

Forty-eight hours pre- and 48 hours post-treatment with statins, the mean relative percentages of PCV2-positive PK-15 cells were significantly increased ($p<0.05$): 3.9 times in atorvastatin (2μM)- and 2.8 times in lovastatin (1μM)-treated cells compared to the mock-treated cells. The mean relative percentages of PCV2-positive PPK cells were also enhanced to 2.4 times (both with 2μM atorvastatin and 1μM lovastatin) compare to their corresponding mock-treated cells. The increased value with atorvastatin (2μM) was statistically significant ($p<0.05$).

Fig. 1. Filipin staining of PK-15 and PPK cells. PK-15 (a, b, c) and PPK cells (d, e, f)) were treated with or without atorvastatin (2μM) and lovastatin (2μM) for 48h before cholesterol labeling was performed using filipin. Magnification 400X.



Discussion

In this study, it was shown that cholesterol depletion from porcine cells by statin treatment resulted in an enhanced level of PCV2 replication. This result is in agreement with the previous findings (2), where it was shown that cholesterol depletion from the plasma membrane of epithelial cells with methyl-β-cyclodextrin strongly enhanced the PCV2 replication. Cholesterol depletion possibly increases the viral internalization and subsequent disassembly of capsid by the disorganization of lipid raft microdomains and the dissociation of proteins bound to lipid rafts (2).

From this study, it can be concluded that depleting plasma membrane cholesterol play a critical role in enhancing PCV2 infection.

Acknowledgements

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P.011

MONOCYTE-DERIVED DENDRITIC CELLS ENHANCE CELL PROLIFERATION AND PORCINE CIRCOVIRUS TYPE 2 REPLICATION IN CONCANAVALIN A-STIMULATED SWINE PERIPHERAL BLOOD LYMPHOCYTES IN VITRO.

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Introduction

Dendritic cells (DCs) are professional antigen presenting cells (APCs) cooperate with other immune cells for the activation of innate and adaptive immune responses (1). Porcine circovirus type 2 (PCV2) is a small, single-stranded, circular DNA virus infecting domestic pigs and causing various PCV2-associated diseases worldwide (2). Previous studies have demonstrated that PCV2 may modulate the innate immune response of DCs, which would render the host more susceptible to concomitant infections (3). On the other hand, replication of PCV2 occurs in mitogen-activated peripheral blood lymphocytes (PBLs) (4). Lymphocyte activation is modulated by various APCs and cytokines (1). To clarify the replication of PCV2 in DCs and/or lymphocytes during their cross talk will bring us a further step to understand the feature of PCV2 infection during the onset of adaptive immune response.

Materials and methods

Swine monocyte-derived DCs (MoDCs) (10^5/well) or PBLs (10^6/well) were inoculated with PCV2 at 1 or 0.1 moi, respectively, in 48-well culture plates for 2 h followed by 2 washes. Bacterial lipopolysaccharide (LPS) at 200 ng/mL and concanavalin A (Con A) at 5 μg/mL were then added to stimulate MoDCs and PBLs, respectively, at the initiation of co-culture. As control, Transwell insert was used to prevent direct cell contact between MoDCs and PBLs. Co-cultured MoDCs and lymphocytes were separated by magnetic cell sorting on incubation days 2-6. PCV2 antigen and genome copy number were determined and quantified by immunofluorescent staining and real time PCR, respectively. Cell proliferation index was measured by 5-(and 6)-carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeling and flow cytometry. To determine the role of cytokines on cell proliferation and PCV2 replication in PBLs, recombinant interleukin (IL)-2, IL-4, and interferon-gamma (IFN-r) were used to stimulate non-Con A-treated, PCV2-inoculated PBLs.

Results

High containing rate without evident intranuclear signals of PCV2 antigen and a slight, but significant, increase in PCV2 genome copy number were detected in PCV2-inoculated MoDCs. The presence of LPS and/or non-Con A- or Con A-stimulated PBLs, however, did not trigger PCV2 replication in MoDCs. In addition, direct contact did not facilitate MoDCs to transmit internalized PCV2 to syngeneic PBLs. On the other hand, active PCV2 replication occurred in PCV2-inoculated, Con A-stimulated PBLs when MoDCs were present. MoDCs induced higher Con A-stimulated proliferation of PBLs (**Table 1A**) and intensified PCV2 replication in infected PBLs (**Table 1B**), for which direct contact between MoDCs and lymphocytes was required. Overall, the level of PCV2 replication and the cell proliferation index were correlated in Con A-stimulated, PCV2-inoculated PBLs. Among the cytokines secreted by Con A-stimulated PBLs, IL-2, but not IL-4 or IFN-r, was mitogenic and induced PCV2 replication in PBLs.

Table 1. Changes in cell proliferation index **(A)** and total PCV2 genome copy number (log) **(B)** in PBLs with time under various treatment conditions. PBLs were inoculated with PCV2. MoDCs were left un-exposed to PCV2 until co-cultured with PBLs.

A)	Days of incubation		
Tx	2	4	6
1	1.07 ± 0.01	1.09 ± 0.01	1.25 ± 0.10
2	1.26 ± 0.32	1.53 ± 0.41	1.65 ± 0.61
3	1.32 ± 0.11	3.04 ± 0.23*	5.21 ± 0.91*
4	1.38 ± 0.10	4.62 ± 1.29**	9.88 ± 2.06**
B)	Days of incubation		
Tx	2	4	6
1	3.93 ± 0.10	3.91 ± 0.31	3.68 ± 0.13
2	4.08 ± 0.23	5.02 ± 0.71	4.57 ± 0.87
3	6.26 ± 0.46*	6.57 ± 0.74*	6.80 ± 0.67*
4	6.81 ± 0.22*	8.22 ± 0.46**	8.26 ± 0.41**

Treatment (Tx): 1). PBLs alone, 2). PBLs + MoDCs, 3). PBLs + Con A, 4). PBLs + MoDCs + Con A
***** Significantly different from Tx 1 on the same incubation day (< 0.05), ** (< 0.01).

Discussion

Immune cells have divergent functions and play different roles on the process of PCV2 replication. As described (3), MoDCs alone support only limited PCV2 replication and potentially are a vehicle of PCV2 only. However, MoDCs could intensify Con A-induced cell proliferation and PCV2 replication in infected PBLs. The present study has demonstrated that the APC function of DCs and IL-2 required for lymphocyte activation/proliferation have significant impacts on PCV2 replication.

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P.012

IN VIVO AND IN VITRO ULTRASTRUCTURAL FINDINGS ON PCV2 INFECTION: A KEY TO UNDERSTAND THE REPLICATION CYCLE

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Introduction

Postweaning multisystemic wasting syndrome (PMWS) was firstly described in the 90s, and linked to porcine circovirus type 2 (PCV2) infection. Since then, a number of studies have been performed to describe the pathological outcome of PMWS and other porcine circovirus diseases (1,2). However, ultrastructural descriptions were performed just as additional data coupling other investigations.

Hence, the main objective of this work was to study the ultrastructural alterations associated to PCV2 infection, both at *in vivo* and *in vitro* levels.

Material and methods

The research was developed in two studies. The first one was on morphogenesis of PCV2 in a clone of the lymphoblastoid L35 cell line (L35 cells), a highly permissive cell line for PCV2 replication. Cells were infected with PCV2, multiplicity of infection of 10, and examined at 0, 6, 12, 24, 48, 60 and 72 hours post-infection (hpi). The second study included the description of ultrastructural findings of mediastinal and inguinal lymph nodes from PMWS affected pigs and healthy control animals.

Both studies were performed by means of electron microscopy (EM) conventional and immunogold labelling techniques. In addition, co-localization studies of PCV2 and mitochondrial antigen with confocal microscopy and double EM immunolabeling were also done.

Results

In the first study, at 0 hpi (2 hours post-incubation of virus and cells), few virion particles were observed either in close proximity to the cell membrane or accumulated as intracytoplasmic inclusions (ICIs) in PCV2-infected cells, close to the plasma membrane and to mitochondria. At 6 hpi, PCV2-infected cells contained larger, double membrane bound ICIs and some mitochondria contained VLPs. Non-membrane bound intranuclear inclusions (INIs) were observed in infected cultures at 12 hpi. At 24 hpi, some cells in the PCV2 infected cultures contained large INIs with VLPs arranged in paracrystalline arrays. Sometimes, INIs containing VLPs were located close to nuclear membrane. At 48 hpi, some PCV2 infected cells showed several and large ICIs with VLPs arranged in paracrystalline arrays, and some ICIs were observed budding from the cytoplasmic cell membrane. At 60 and 72 hpi, all changes were similar to those described above. The number of dead cells increased over time.

For the second study, significant ultrastructural alterations were noted mainly in histiocytes infiltrating lymphoid tissues from diseased animals only. Severe swelling and proliferation of mitochondria, and proliferation and dilation of rough endoplasmic reticulum and Golgi complex were the most significant findings. An important number of ICIs were observed in close proximity with those damaged organelles and labelled to PCV2. Viral factories (VFs) were often found next to swollen mitochondria.

PCV2 and mitochondria antigens were co-localized frequently both *in vivo* and *in vitro*.

Discussion

The presented *in vitro* data of PCV2 replication together with previously published information using other cell models (3,4), and the findings of the *in vivo* study, allowed proposing a sequence of events that may occur during PCV2 replication cycle.

PCV2 is internalized by endocytosis, and then aggregated in intracytoplasmic inclusion bodies (ICIs). Later, VLPs are closely associated with mitochondria, completing a first cytoplasmic phase. Next, the virions get into the nucleus for replication, assembly and encapsidation, with the nuclear membrane participation. Immature virions leave the nucleus and form ICIs in a second cytoplasmic phase, with organelle membranes participation. At the end of the replication cycle (24-48 hours), PCV2 particles are released by: a) budding of mature virion clusters, b) exocytosis of free VLPs or, c) lysis of apoptotic/death cells. Obtained results allowed to complement existing works on the PCV2 replication cycle in cell culture and to gain new insights on the pathogenesis of PMWS. The description of the intimate association between PCV2 and mitochondria is one of the major contributions of the present research, as well, for the first time, a proposal of PCV2 morphogenesis.

Acknowledgements

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P.013

MODULATION OF INNATE IMMUNE RESPONSE IN BLOOD MONOCYTES FROM CLINICALLY HEALTHY ASYMPTOMATIC PORCINE CIRCOVIRUS TYPE 2 (PCV2)-CARRIER PIGS FOLLOWING RE-EXPOSURE TO PCV2 AND/OR LIPOPOLYSACCHARIDE STIMULATION IN VITRO

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Introduction

In Taiwan, most pig farms display >90% of serum-positive and nucleic acid detection rates for PCV2. However, the majority of PCV2-infected pigs remain asymptomatic with only some developing disease symptoms. The objective of the present study was to characterize and compare the development of initial innate immune response following PCV2 re-exposure and/or LPS stimulation in blood monocytes (Mos) *in vitro* between clinically healthy, asymptomatic, PCV2-carrier and PCV2-free SPF pigs. Treatments such as PCV2 re-exposure and/or LPS stimulation were selected to mimic recurrent viral infection and/or Gram-negative bacterial infection. The expression levels of mRNA of 24 genes including toll-like receptor (TLR)-1 to -10 and, interferon-regulatory factor (IRF)-1, -3, -6, -7, -9 and NF-κB and various pro-inflammatory cytokines and interferon, including IL-1, IL-6, IL-8, IL-10, IL-12p35, IL-12p40, TNF-α, and IFN-α were evaluated.

Materials and methods

Animals and cells: Four 10- to 14-week-old, conventional pigs containing 10^0-10^1 copies of PCV2 genome/10^6 Mos (PCV2-carrier pigs) and 3 age-matched PCV2-free SPF pigs were used for blood collection and Mos isolation. **Virus:** The PCV2 isolate was from a PMWS-affected pig. **Experimental design:** The mock-, PCV2-, LPS-, and PCV2/LPS-treated Mos were included. The PCV2 inoculum was 0.1 moi and the conc. of LPS (*E. coli*) was 1 μg/ml. Following 3 days of incubation, the mRNA expression levels of the 24 genes were measured by relative q-rt-PCR. **Relative quantification of gene expression by q-rt-PCR:** The total RNA was extracted and the first-strand cDNA was reversely transcribed from 1 μg of total RNA. The cDNA was amplified by q-rt-PCR using specific primer pairs and the relative quantification of the gene expression was performed by the $2^{-\Delta\Delta Ct}$ method using G3PDH and β-actin as reference genes (RG). The ΔΔCt values = $[(CtTG\text{-}CtRG)_{treat}]\text{-}[(CtTG\text{-}CtRG)_{mock}]$, where CtTG is the threshold cycle (Ct) of the target gene (TG), CtRG is the geometric mean of Ct of the RG.

Results

A general down-regulation in TLRs was seen in carrier pigs except for TLR-7, -9, and -10 as well as TLR-3, -5, -7, and -10 in the PCV2- and PCV2/LPS-treated groups, respectively.

Up-regulation of IRF-7 was seen in PCV2-treated carrier pigs. However, the expression of IFN-α was not consistent with the up-regulation of IRF-7 in PCV2-treated carrier pigs as anticipated.

For carrier pigs, IL-1α, IL-6, IL-8, IL-10, and TNF-α, were up-regulated and on the contrary for SPF pigs, a general down-regulation was seen in most cytokine genes in the PCV2-treated group.

Gene	Treatment					
	PCV2		LPS		PCV2/LPS	
	Carrier	SPF	Carrier	SPF	Carrier	SPF
TLR-1	-1.13	-1.23[a]	-1.18*	-2.48*, [b]	-1.13*	-2.36*, [b]
TLR-2	-1.6*	1.07*	-1.32	-1.33	-1.74	-1.31
TLR-3	-1.3	-1.18	-1.73	-1.63	1.25	-2.49
TLR-4	-1.13	1.06[a]	1.01*	-2.04*, [b]	1.00*	-1.98*, [b]
TLR-5	-1.74	1.02	-1.22	-1.39	1.55	-2.26
TLR-6	-1.64	1.09	-1.40	-1.18	-1.21	-1.51
TLR-7	1.08	-1.05	-1.04	-2.91	1.07	-1.65
TLR-8	-1.70	1.15	-1.84	-1.87	-1.44*	-3.92*
TLR-9	2.08[a]	-1.47	-1.81[b]	-2.13	-1.35[b]	-1.28
TLR-10	1.74	1.11	-1.03	-2.04	1.58*	-2.49*
IRF-1	-0.88	-1.4	1.78*	-1.75*	1.49	-1.24
IRF-3	-1.20	-1.6	-1.14	-1.55	1.07	-2.4
IRF-6	-1.54	-1.35	-1.70	-1.68	1.07	-1.97
IRF-7	1.71	-1.03	-1.05*	-2.46*	1.33*	-2.29*
IRF-9	-1.39	-1.01	-1.31	-1.38	1.00	-1.73
NF-κB	1.73[a, b]	-1.05	2.89[a]	1.16	1.5[b]	1.55
IL-1α	1.98[b]	-1.22[b]	5.58[a]	6.96[a]	5.77[a]	6.68[a]
IL-6	8.65	[&]0	7.1	0	13.69	0
IL-8	1.85*, [b]	-2.23*, [b]	5.38[a]	6.42[a]	5.3[a]	5.77[a]
IL-10	1.92	-1.19	2.35	1.31	2.85*	1.64*
IL-12p35	-1.12	-1.08	1.14	-1.32	1.44*	-2.25*
IL-12p40	-1.26	-1.20	-1.19	1.27	-1.24	-1.66
TNF-α	1.49	-1.39	1.66	2.30	1.64	-1.05
IFN-α	-1.27	-1.31	-1.25	-1.31	1.55	-1.74

[&] The mRNA expression level is below detection limit. *The difference between SPF and carrier pigs is significant ($p < 0.05$). [a, b, c] Different labels in the same gene among different treatment groups differ significantly ($p < 0.05$).

Discussion

To certain danger signals, the PCV2-containing Mos of carrier pigs indeed displayed altered responsiveness from that of PCV2-free Mos of SPF pigs. The alterations included down-regulated mRNA expressions of IFN-α, most TLRs, and IRFs when encountering the secondary viral or bacterial stimulation; however, the mRNA expressions of NF-κB and most related pro-inflammatory cytokines were up-regulated. It is speculated that dis-regulation in IRF-7-involved IFN-α production pathway, instead of IRF-7 directly, is responsible for the reduced PCV2-conducted IFN-α production and IFN-α-mediated innate immune responses. The altered immune status of the asymptomatic PCV2-carrier pigs may make them more susceptible to the further development of PCV2-associated diseases following recurrent PCV2 and/or concomitant other viral or bacterial infection.

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P.014

ANTIGENIC DIFFERENCES AMONG PCV2 STRAINS OF DIFFERENT GENETIC CLUSTERS AS DEMONSTRATED BY THE USE OF MONOCLONAL ANTIBODIES GENERATED AGAINST PCV2A OR PCV2B

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Introduction

Porcine circovirus type 2 (PCV2) is the causal agent of postweaning multisystemic wasting syndrome (PMWS) in weaned pigs (1). A recently proposed classification system divides the PCV2 strains into two major genotypes (PCV2a and PCV2b) and 8 genetic clusters: 1A to 1C (PCV2b) and 2A to 2E (PCV2a) based on their genomic sequences (2, 3). Lefebvre et al. (4) confirmed antigenic differences between different PCV2 strains by using mouse monoclonal antibodies (mAbs) raised against PCV2a. In that study, only a limited number of PCV2 strains with only 3 out of 8 genetic clusters were enclosed. To date, no studies have been performed to identify antigenic differences between different PCV2 strains including all genetic clusters of both genotypes PCV2a and PCV2b by using mAbs raised against both genotypes PCV2a and PCV2b. Thus, the present study aimed to identify antigenic differences between PCV2 strains of all genetic clusters by using mAbs raised against both PCV2a and PCV2b.

Materials and Methods

Fourteen different PK-15 cell-adapted PCV2 strains of all eight PCV2 clusters and with different clinical and geographical origin were used in this study. 1A/1B strains: 48285, 1147, II9F, 1206; 1C: NL_Control_4, II11A; 2A: Aust 10; 2B: Pingtung-1 and Pintung-4; 2C: PCV2 390 (kindly provided by Dr. Monica Balasch); 2D: Tomasz (isolated in our lab) and 2E: Stoon-1010, 1121 and 1103 were enclosed.

Sixteen mouse monoclonal antibodies (mAbs) (9C3, 13H4, 16G12, 21C12, 31D5, 38C1, 43E10, 48B5, 55B1, 59C6, 63H3, 70A7, 94H8, 103H7, 108E8 and 114C8) were generated against genotype PCV2a strain Stoon-1010 (4) and 6 mouse mAbs (6E9, 12E12, 14G2, 19C1, 19G10 and 22C1) were generated against genotype PCV2b strain 1147. All 14 PCV2 strains mentioned above were used to make 96-well IPMA plates as described by Saha et al. (5). PCV-negative PK-15 cells and the persistently PCV1 infected PK-15 cell line were used for control IPMA plates. The staining procedure was similar to the IPMA technique described by Lefebvre et al. (4) with ten-fold serial dilutions of hybridoma supernatants (mAbs) in PBS used as primary Abs. IPMA antibody titres of a hybridoma supernatant were expressed as the reciprocal of the last dilution that resulted in a positive reaction. These assays were performed independently for 3 times for each strain.

Results

Four out of 22 mAbs reacted with all PCV2 strains with the titres ranging from 10 to 10,000 (12E12, 21C12, 38C1 and 114C8). One mAb, 19G10 stained all PCV2 strains except the strain 390 which belongs to cluster 2C. MAbs 6E9, 9C3, 16G12, 43E10, 55B1, 63H3, 70A7, 94H8 and 103H7 did not react with the PCV2 strains of clusters 1C, 2B, 2C and 2D or they had IPMA Ab titres to these strains at least 100 times lower than for the PCV2 strains of clusters 1A/1B, 2A and 2E. Only two mAbs (14G2 and 19C1) were found to be specific for 1A/1B strains, although mAb 19C1 showed very low IPMA antibody titres (1 to 10) to 1A/1B strains. MAbs 31D5, 59C6 and 108E8 had titres ranging from 1,000 to 10,000 for 2A and 2E strains and did not react to the other strains or they had IPMA antibody titres at least 100 times lower than for the 2A and 2E strains. MAb 22C1 did not react or showed very weak reaction with PCV2a strains (2A to 2E) but clearly reacted with all PCV2b strains (1A to 1C, except strain NL_Control_4) and IPMA antibody titres were at least 100 times higher to PCV2b strains than for the PCV2a strains. MAb 13H4 stained only Stoon-1010 (PCV2a 2E) but did not stain or had very low titres to all other PCV2a or PCV2b strains. MAb 48B5 reacted with all PCV2a strains (except 390, type 2C strain) but only one PCV2b strain, 1206 (type 1A/1B). None of the 22 mAbs reacted with PCV1 infected cells or PCV-negative PK-15 cells.

Discussion

This study demonstrates that the different genetic clusters can be discriminated by using a large panel of mAbs. The findings might have consequences for the development of diagnostic assay. Four universal mAbs were reactive to all PCV2 strains. Some mAbs were cluster-specific. The results of this study may be indicative for possible binding sites of different mAbs in the capsid protein.

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P.015
MAPPING OF NEUTRALIZING EPITOPES IN THE CAPSID PROTEIN OF PORCINE CIRCOVIRUS 2

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Introduction

PCV2 is the causative agent of Porcine Circovirus Associated Diseases (PCVAD) with postweaning multisystemic wasting syndrome (PMWS) as the most significant manifestation (1). PMWS-affected pigs have significantly lower levels of PCV2-neutralizing antibodies when compared to sub-clinically infected animals (2), suggesting a crucial role for neutralizing antibodies in the prevention of PMWS. Previously, Lefebvre et al. (3) demonstrated that monoclonal antibodies (mAbs) 16G12, 38C1, 48B5, 63H3 and 94H8 against the PCV2 capsid protein neutralise strains Stoon-1010 (genotype 2a) and 48285 (genotype 2b) while strains 1121 (genotype 2a), 1147 and VC2002-k39 (both genotype 2b) are not neutralized. By using the neutralizing mAb 3F6 in a Pepscan, Shang et al. (4) have identified linear neutralizing epitopes at amino acid (aa) positions 145-162 and 175-192 of the PCV2 capsid protein, but the particular contribution of specific aa residues in the PCV2 neutralizing epitope(s) has not been determined yet. The present study aimed to identify aa residues that contribute to the PCV2 neutralizing epitope(s) by using specific mAbs in virus neutralization tests and by site-directed mutagenesis of infectious clones and by reactivity of neutralizing mAbs and porcine hyperimmune serum with capsid proteins and fragments thereof in PK-15 cells.

Materials and Methods

Amplification of the genomes of Stoon-1010 and 48285 was performed to construct infectious clones. Infectious clone II9F-13 from strain VC2002-k39 was described in the past (5). Previously, alignment of the capsid proteins (3) has shown a very limited number of aa differences between the strain Stoon-1010 that can be neutralized by mAbs (N+) and the strain 1121 that cannot be neutralized by mAbs (N), and between strain 48285 (N+) and the strains 1147 and VC2002-k39 (both N-). Therefore, these aa residues were selected for site-directed mutagenesis.

The capsid gene of the infectious clone 48285-24 was mutated using primers 48285TtoA1147FW and 48285TtoA1147REV at aa position 190 (from T to A); Stoon-1010-8 was mutated at position 191 (from E to R) using primers StoonEtoR1121FW and StoonEtoR1121REV and at aa position 131 (from T to P) using StoonTtoP1121FW and stoonTtoP1121REV; II9F-13 was mutated at aa position 190 (from A to T) using primers II9FAtoT48285FW and II9FAtoT48285REV and at aa position 151 (from P to T) using primers II9FPtoT48285FW and II9FPtoTREV48285.

Mutagenesis at both positions of Stoon-1010-8 and II9F-13 was also performed.

Sensitive neutralization assays (SN) were performed with parental infectious clones and site-directed mutated infectious clones by using 5 neutralizing mAbs (16G12, 38C1, 48B5, 63H3 and 94H8) (3).

The full-length capsid (233 aa), N-terminal fragment (coding for aa 1-80), internal fragment (coding for aa 75-160), C-terminal fragment (coding for aa 161-233), and two more capsid fragments coding for aa 1-160 and aa 75-233 of Stoon-1010-8 were amplified by PCR and cloned in an expression vector. Full length or partial capsid fragments were transfected into PCV negative PK-15 cells and PCV2 proteins were visualized by immunofluorescence staining using neutralizing mAbs and porcine hyperimmune serum raised against Stoon-1010.

Results

Mutation of aa position 190 (TtoA) in 48285-24 resulted in a loss of neutralization. A single mutation at aa position 131 (TtoP) or 191 (EtoR) in Stoon-1010-8 resulted in reduced neutralization and the double mutant showed more reduction in neutralization than the single mutants. Mutations at aa position 151 (PtoT), 191 (AtoT), or both in VC2002-k39 resulted in a gain of neutralization.

A full-length Stoon-1010 capsid (1-233 aa) and several fragments (aa 1-80, 1-160, 75-160, 75-233, 161-233) were expressed in PK-15 cells. Immunofluorescence stainings with mAbs and porcine hyperimmune serum showed strong coloration only with the full-length capsid.

Discussion

In this study, several amino acids (aa positions 131, 151, 190 and 191) dispersed throughout the capsid protein were identified as a part of (a) neutralizing epitope(s). Our transfection experiment suggests that this neutralizing epitope is discontinuous and conformational.

Acknowledgements

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P.016

MATERNAL PORCINE CIRCOVIRUS TYPE 2-MEMORY T CELLS TRANSFERS TO PIGLETS THROUGH COLOSTRUMS INGESTION: COMPLETED STUDY

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Introduction

Passive transfer from colostrum plays a crucial role in initial protection of new-born piglets from pathogens as no transfer of maternal immunity occurs in foetal life, due to the characteristic placenta structure of pigs. Colostrum provides an essential source of maternal antibodies for piglets. Since the late 80s, reports indicate transferral of lymphocytes to piglets through colostrum (1, 2). Recently, Antigen –specific maternal interferon gamma-producing lymphocytes are shown to be transferred to piglets (3, 4, 5, 6). Their phenotypes have not been fully elucidated yet. Because maternal lymphocyte transferred from colostrums provides a protection against the pathogen to which sows have established specific immunity (5), it is highly expected that antigen-specific memory lymphocytes transfer to piglets through colostrum. Memory T cells immediately proliferate against the second infection from the primed-pathogen. Hence, we have confirmed that blood T cells in porcine circovirus type 2 (PCV2)-vaccinated sow rapidly proliferates after in vitro stimuli of primed antigens, which mimic second infection. Preliminary results from the same study on a limited number of sows were presented earlier (7).

Materials and Methods

CIRCOVAC®, the maternal vaccine for PCV2 from Merial Japan was used in this study as this vaccine was preliminarily confirmed as inducing memory T cells in blood. 6 sows were used in this study; half were vaccinated twice with CIRCOVAC, 2mL IM, 5 and 2 weeks before delivery, and the other were left as a non-vaccinated control. 5 piglets were randomly selected from each of the vaccinated-sows and defined as a PCV2-vaccinated group. 5 piglets from each of the non-vaccinated sows were regarded as a control group. Heparinized-blood from sows was collected before and after delivery, and also collected from piglets in both groups at 3 days, 7 days, 14 days and 21 days after birth. The blood was stimulated with CIRCOVAC in vitro for 6 hrs to mimic second infection. After a 6-hour incubation, T cells in the blood were stained with FITC-labeled anti-porcine CD3 antibody and then the rate of $CD3^{high}$ cells, T cells under further maturation (8), in peripheral blood lymphocyte (PBL) was analyzed with a FACSCalibur flow cytometer. The $CD3^{high}$-T-cell proliferate index (TCI) was calculated as follows; TCI = the rate of $CD3^{high}$ T cells in CIRCOVAC-stimulated PBL / the rate of $CD3^{high}$ T cells in unstimulated PBL. Data were statistically analyzed using Repeated measure ANOVA.

Results

TCI of vaccinated-sows was 5.09 before delivery and 2.72 after, whereas the non-vaccinated sows showed 1.03 and 0.92 before and after delivery respectively. This suggests that vaccination successfully induced memory T cells against PCV2 in the blood. TCI of piglets are presented in Table 1. TCIs of PCV2-vaccinated group were significantly higher than those of control group ($p<0.01$).

Table 1: TCI of piglets

Age	3 days	7 days	14 days	21days
Vaccinated group(n=15)	2.90± 1.93*	2.88± 1.26*	3.21± 1.70*	2.26± 1.22*
Control group(n=15)	1.01± 0.12	1.26± 0.47	1.00± 0.05	1.00± 0.04

* $p<0.01$

Discussion

In conclusion, this study suggested that not only antibodies but also memory T cells transfers through colostrum in pigs. This phenomenon should be critical for protection of new-born piglets from initial infections. Further study is now being conducted using increased number of sows.

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P.017

EFFECTS OF A PORCINE CIRCOVIRUS TYPE 2 (PCV2) SUBLINICAL INFECTION UPON IMMUNOLOGICAL PARAMETERS FOLLOWING AN AUJESZKY'S DISEASE VIRUS VACCINATION

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Introduction

Porcine circovirus type 2 (PCV2), the causative agent of postweaning multisystemic wasting syndrome, is reported to have marked immunoregulatory capabilities for the immune system of pigs (1). Previous research showed that PCV2-infected peripheral blood mononuclear cells are impaired in their responses to mitogens (2). Also, dendritic cells and macrophages infected *in vitro* by PCV2 have altered immune capabilities (1). This ability of PCV2 to down-regulate the immune response of the pig raises the question of whether or not PCV2 infection may interfere with the efficacy of other vaccines used in pigs under field conditions. Therefore, the aim of the present work was to clarify if a PCV2 subclinical infection could affect the development of specific immunity against an Aujeszky's disease virus (ADV) vaccination.

Materials and methods

Twenty one two week–old conventional piglets, sero-positive to PCV2 and negative to PCV2 PCR, were used. At six weeks of age, piglets were randomly distributed into four separated groups and were inoculated with PCV2 or placebo (Table 1). Fourteen days later, pigs were vaccinated with a gE^- tk^- attenuated ADV vaccine (Bartha K61). Clinical course and weight gains (ADWG) were measured from 0 to 35 days post-inoculation (dpi). At 35 dpi pigs were necropsied and histopathological studies as well as *in situ hybridization* (ISH) (3) were performed. Blood samples were taken weekly and used to determine PCV2 viral load by means of a quantitative PCR (qPCR) (4), anti-PCV2 antibodies (IPMA), total anti-ADV antibodies (Ingezim ADV total, Ingenasa) and ADV-neutralizing antibodies (NA) (OIE protocol). Cell mediated immunity against PCV2 and ADV was measured by means of the IFN-γ ELISPOT. Statistical analysis was done using Statsdirect v2.7.8.

Table 1. Study planning and treatment distribution

	Days post-inoculation		
Group	**0 dpi**	**14 dpi**	**35 dpi**
IC (n=4)	PCV2[a]	Placebo	Necropsy
IV (n=7)	PCV2[a]	ADV vaccine[b]	Necropsy
CC (n=4)	Placebo	Placebo	Necropsy
CV (n=6)	Placebo	ADV vaccine[b]	Necropsy

[a] Inoculated with 10^4 $TCID_{50}$/pig of PCV2b.
[b] Vaccinated with ADV live attenuated gE^- tk^- vaccine (Auskipra®, Lab. Hipra). IC: infected unvaccinated; IV: infected vaccinated; CC: control; CV: control vaccinated.

Results

No relevant clinical signs or gross and microscopic lesions were observed in PCV2-inoculated pigs. Although all PCV2-inoculated pigs developed viremia, only one animal (IC group) was still positive for PCV2 by ISH at 35 dpi. ADWG from 0 to 14 dpi and from 21 to 28 dpi were lower in PCV2-inoculated pigs compared with non-inoculated ones ($p<0.05$). Also, PCV2-inoculated pigs seroconverted by 14 dpi, reaching an average IPMA titer of $13\log_2$ at 35 dpi. PCV2-specific IFN-γ ELISPOT responses were noticeable from 21 dpi onwards in PCV2-inoculated pigs compared to non-inoculated ones ($p<0.05$). No differences between inoculated groups (IC and IV) were observed for IPMA titers or IFN-γ ELISPOT.

All pigs had no ADV antibodies at the starting of the experiment. By 35 dpi (3 weeks post-ADV vaccination) ADV NA were found in all vaccinated animals but one from group IV; no significant differences in ADV NA between CV and IV were observed. Both groups showed significant ADV-specific IFN-γ responses in ELISPOT as early as 21 dpi (7 days post-vaccination). No differences were observed in ADV-specific IFN-γ responses between CV and IV.

Discussion

In vitro and *in vivo* studies indicate that PCV2 interferes with the innate and acquired immunity as well as with the functionality of antigen presenting cells (1). The hypothesis underlying the present work was the fact that a PCV2 subclinical infection may interfere the development of immunity after vaccination against a given antigen. In the purpose of this latter point, the effect of a PCV2 subclinical infection upon ADV vaccination in pigs was assessed. In the present study, immune responses after ADV vaccination (measured by NA and interferon responses) were not significantly affected by a subclinical PCV2 infection.

Acknowledgements

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P.018

CHARACTERIZATION OF THE CELLULAR IMMUNE RESPONSE TO PCV2B: IMPLICATIONS FOR POST-WEANING MULTISYSTEMIC WASTING SYNDROME

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Introduction

Post-weaning multisystemic wasting syndrome (PMWS) is a global endemic disease with extremely high economic impact and severe consequences for welfare of pigs. Porcine circovirus type 2 (PCV2) has been identified as the essential infectious agent of PMWS. Compelling evidence suggests that the interaction between PCV2 and the immune system is a key event in the pathogenesis of PMWS. However, little is known about the mechanisms utilized by the virus to persist in clinically normal pigs or the factors triggering PCV2 replication.

The objective of the present work was to determine the gene-expression profile and biological pathways involved in a subset of immune cells in response to PCV2 infection.

Materials and methods

Six PCV2 PCR- and AB-negative Large White × Landrace pigs (*Sus scrofa*) were sacrificed and alveolar macrophages (AMΦs), monocyte-derived dendritic cells (MoDCs) and bone-marrow cells (BMCs) generated. The current dominant UK PCV2b isolate was derived from farms severely affected with PMWS, and subsequently characterized and propagated. All three cell types were either incubated with PCV2b (MOI of 0.5 TCID50) or mock inoculum for 0h, 1h or 24h. After incubation, Cy3-labeled cRNA was prepared and hybridized to the Porcine Gene Expression Microarray (V2; Agilent). Following quality control, differentially expressed genes were analyzed using R. Differences in gene expression between PCV2b-infected cells and mock-treated controls were determined using a cut-off -2 ≤ fold change ≥ 2. Gene Ontology (GO) based functional interpretation of the data was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) (1).

Results

All three cell types were highly susceptible to PCV2 infection and showed differential gene expression at an early (1h) and late timepoint (24h).
Alveolar macrophages (AMΦs) showed the most marked response to PCV2b. Early response to PCV2b lead to the upregulation of several immune-associated genes and cytokines including complement C3, TNFa and IL23a.

At 24h post infection (pi), the vascular cell adhesion molecule 1 (VCAM-1) along with several interferon-regulated antiviral response genes ISG15 and Mx1 were shown to be upregulated in PCV2-infected alveolar macrophages.

Table 1. Differentially-expressed transcripts between PCV2- and mock-infected alveolar macrophages at 1h and 24h pi (N=6)

Comparison	Fold change	Gene name	Product
PCV2 1h vs mock 1h	2.864	TNFa	tumor necrosis factor alpha
PCV2 1h vs mock 1h	2.228	HMOX1	heme oxygenase 1
PCV2 24h vs PCV2 1h	-3.263	-	chemokine ligand 24-like protein
PCV2 24h vs PCV2 1h	-2.677	IL23A	interleukin 23, alpha subunit p19
PCV2 24h vs PCV2 1h	-2.055	C3	complement component 3
PCV2 24h vs mock 24h	2.133	MX1	myxovirus (influenza virus) resistance 1
PCV2 24h vs mock 24h	2.598	VCAM1	vascular cell adhesion molecule
PCV2 24h vs mock 24h	2.093	ISG15	ISG15 ubiquitin-like modifier

Discussion

The present study provides further insight into the early and cell type-specific response to PCV2b infection. All immune cell subsets used were easily infected by PCV2b *in vitro*, thus potentially highlighting their role *in vivo*. One of the most compelling findings was an upregulation of the interferon-regulated antiviral response genes ISG15 and Mx1 in alveolar macrophages 24 h pi. Antiviral activity of ISG15 is associated with protein ISGylation *in vitro* and/or *in vivo*, and has been reported for both, DNA and RNA viruses. Interestingly both, ISG15 and MX1, have recently been shown upregulated in mediastinal lymph nodes (MLNs) and lyzed whole blood (LWB) of subclinically-infected pigs a few days after PCV2 infection (2). The universal expression of these genes in MLNs, LWB and alveolar macrophages, may make them potential targets for novel strategies to improve disease outcome upon PCV2 infection.

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P.019

THE IMPACT OF PORCINE CIRCOVIRUS TYPE-2 INFECTION ON MALE REPRODUCTIVE ORGANS OF BOARS

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Introduction

Porcine circoviruses type 2 (PCV2) causes multisystemic diseases in pigs involving respiratory problems, immunological dysfunctions and reproductive failures currently called porcine circovirus associated diseases (PCVAD). Abortion with the presence of PCV2 in aborted fetuses confirms PCV role in sow reproductive failure (1). PCV2 could also be detected in semen of experimentally infected boars (2). Experimental insemination with PCV2-contaminated semen could also induce reproductive failure in pregnant sows (3). However, an impact of PCV2 infection on reproductive organs of boars is yet being elucidated. Therefore, we aimed to investigate the association of PCV2 infection on the pathological lesions occurring in the reproductive organs of boars.

Materials and methods

The reproductive organs of culled boars (n = 38) including testes, epididymides, seminal vesicles and superficial inguinal lymph nodes were collected from slaughter houses in western part of Thailand. The organs were grossly examined and collected for histopathology and the inguinal lymph nodes were subjected to PCV2 polymerase chain reaction (PCR) test using the primers previously published (4).

According to prominent macroscopic lesions of the male reproductive organs, samples were separated into two groups; Group 1 having edema of the epididymal heads (n = 20) and Group 2 grossly look normal on the epididymal heads (n = 18).

Results

The results revealed many interesting lesions found in the group having edema of the epididymal heads including lymph node enlargement (4/20, 20%), lymphoid depletion (5/20, 25%), testicular fibrosis (100%, 20/20), testicular degeneration (15/20, 75%). Interestingly, in the non-edema group, the lymph nodes were normal in size but a few having lymphoid depletion (4/18, 22.22%). Testicular fibrosis was less seen (12/20, 66.67%) but similar numbers were observed for testicular degeneration (14/18, 77.78%). PCR revealed positive results for PCV2 in both groups with 45% (9/20) in group 1 and 72.22% (13/18) in group 2, respectively.

Discussion

The striking lesion found in the boar reproductive organs was edema of the epididymal head.

Fig. 1: Severe bilateral edema of the epididymal head from the boar (no. R91) with natural PCV2 infection.

This edematous lesion may be associated with PCV2 infection as it was found in boars with PCV2 infection. Edema of epididymis particularly seen at the head of epididymis and spermatic cord has previously been reported in a boar with PCV2 infection (5). However, only 45% of those pigs with epididymal edema were positive for PCV2 detection while 72.22% of pigs in the other group were positive for PCV2. Our data indicated that edema of the epididymides does not always cause by PCV2 infection in boars. However, more tests are needed to support these observations. IHC should be done to confirm if the PCV2 antigen was present in the reproductive organs. Detecting PCV genome from the inguinal lymph nodes may not reflex on the reproductive lesions.

Acknowledgements

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P.020

EXTRACELLULAR MATRIX COMPONENTS IN LYMPHOID ORGANS OF HEALTHY AND POSTWEANING MUTISYSTEMIC WASTING SYNDROME (PMWS)-AFFECTED PIGS

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Introduction

The extracellular matrix (ECM) is a complex network of macromolecules that, besides its structural role, it participates in a variety of cellular functions. For example, in lymphoid tissues the ECM is involved in lymphocyte migration, differentiation and activation. However, little is known about the composition of ECM in porcine lymphoid organs. Furthermore, increasing evidence suggests that the ECM is also actively involved in pathologic conditions, such as infection and inflammation (1).

Postweaning multisystemic wasting syndrome (PMWS) is a multifactorial disease for which porcine circovirus type 2 (PCV2) is considered the essential infectious agent. The major histopathological lesions are generalized lymphocyte depletion with histiocytic infiltration in lymphoid organs (2).

The aim of this work was to define the normal distribution of different components of the ECM, hyaluronan (HA) and its receptor CD44, the HA-binding proteoglycan versican and the glycoprotein tenascin-C (TN-C) in porcine lymphoid organs and to study how the expression pattern of these molecules was altered in PMWS-affected pigs.

Materials and methods

Formalin-fixed, paraffin-embedded samples corresponding to lymphoid organs (including lymph node, thymus and tonsil) from both healthy (n=5) and PMWS affected (n=5) (3) pigs were used in the study. The expression of HA was assessed by histochemistry using a HA binding protein. CD44, versican and TN-C were analyzed by immunohistochemical procedures using the corresponding monoclonal antibodies.

Results

Control pigs:

In lymph nodes and tonsil, HA was expressed both in follicles and T-dependent areas, showing a preference for follicular structures. The basal layer of tonsillar epithelium, the underlying connective tissue and surrounding crypts tissue also stained for this molecule. In the thymus HA was mainly present in the medulla, whereas in the cortex was restricted to punctual areas. The connective tissue of the capsula, *septae* and *trabeculae* of these organs was highly positive for HA.

CD44 was observed in the parafollicular area and also in some specific cells inside the follicles. As for HA, CD44 was basically confined to the thymic medulla. Versican was hardly detected in the studied tissues, being restricted to some vascular structures and some large *trabeculae*. Certain expression of versican was also observed in the basal membrane of the tonsillar epithelium.

In lymph nodes and tonsil, TN-C was variably expressed through the parafollicular zones, whereas the follicles were devoid of TN-C. Again, structures such as the capsula, *septae* and *trabeculae*, as well as the tonsillar subepithelial connective tissue and tissue surrounding crypts had a positive result for TN-C staining. As the other molecules, TN-C was mainly located in the thymic medulla, with punctual signalling in the cortical area.

PMWS-affected pigs:

All lymphoid tissues studied displayed moderate to servere lymphocyte depletion. In these samples the normal structure of the follicles was lost. HA had a homogeneous distribution pattern through all the tissue. Even though the localization of CD44 did not change, there was a slight decrease in its expression in the parafollicular areas. Versican was found to be punctually increased in the connective tissue in some samples.

The most significant difference was observed in the expression of TN-C TN-C staining raised in the parafollicular areas and in the tonsillar crypts. The connective tissue was the most strongly marked region for this molecule. No pathological thymic tissue was available for this study.

Discussion

This study describes for the first time the location of HA, CD44, versican and TN-C in lymphoid organs of healthy and diseased animals, indicating that the pattern of expression of these molecules was altered in PMWS-affected pigs. These changes could be related to lymphocyte depletion. However, further studies are needed to define the precise role of each molecule in the pathogenesis of the disease.

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P.021

EXTRACELLULAR MATRIX MOLECULES IN THE ILEUM OF POST-WEANING MULTISYSTEMIC WASTING SYNDROME- AND PORCINE PROLIFERATIVE ENTEROPATHY-AFFECTED PIGS

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Introduction

Hyaluronan (HA) and other extracellular matrix (ECM) molecules, such as proteoglycans like versican or the glycoprotein tenascin-C (TN-C), have been increasingly involved in the regulation of inflammation. HA participates in leukocyte recruitment via interaction with CD44 and activates various inflammatory cells and signals through TLR receptors. In different cell types, the viral mimic poly(I:C), certain viruses and other inflammatory stimuli induce organization of HA into cable-like structures, which are responsible for leukocyte adhesion and might modulate their activation (1).

Infectious diseases are of great importance for pig industry due to economic losses. The present study focused in two diseases: porcine proliferative enteropathy caused by the obligate intracellular bacterium *Lawsonia intracellularis* (2), and postweaning multisystemic wasting syndrome (PMWS) for which porcine circovirus type 2 (PCV2) is considered the essential infectious agent (3). The goal of this work was to study the involvement of HA, CD44, versican and TN-C in mentioned diseases using *in vivo* and *in vitro* studies.

Materials and methods

In vivo: Formalin-fixed, paraffin-embedded ileum samples from healthy pigs (n=5), pigs with proliferative enteropathy (n=4) and with PMWS (n=5) were obtained from the Veterinary Pathology Diagnostic Service (UAB). The expression of HA, CD44, versican and TN-C was analyzed by histochemical and immunohistochemical procedures.

In vitro: Two porcine epithelial cell lines (IPEC-J2 and IPI-2I) and primary smooth muscle cell (SMC) cultures from the ileum of healthy pigs were treated with poly(I:C). Changes in HA expression were analyzed by cytochemistry.

Results

In control pigs, HA was abundant in the *lamina propria*, connective tissues and in the germinal center and capsule of Peyer's patches. CD44 was especially abundant in the dome of the Peyer's patches, the region closer to the mucosa. Epithelial cells did also stain for CD44, mainly in the crypts. No versican immunoreactivity was detected in any sample. TN-C was mainly expressed in points of the basal membrane and in the muscular layer.

In all proliferative enteropathy samples, a decrease in HA was observed in the Peyer's patches as well as in the crypts and the *lamina propria*, although it increased in the submucosa. CD44 expression also decreased and epithelial cells were negative. In contrast, versican was increased and present in specific spots in the basal membrane and the connective tissue between the muscular layers. The most remarkable changes regarded TN-C, which was abundantly expressed in the submucosa and increased in the basal membrane, muscular layer and *lamina propria.*

PCV2 infection was characterized by lymphocyte depletion of Peyer's patches. It was noticed a decrease in HA content, which remained in a badly structured pattern, and displayed a change in CD44 localization in the most severe cases, being expressed through all the *lamina propria*. Versican was also found in the basal membrane, although its expression was variable. Compared to controls, TN-C increased in the submucosa, especially around Peyer's patches.

In vitro results showed changes in the HA staining pattern when cells were treated with poly(I:C), observing an increase in the fraction of HA associated to the cell. In SMCs, the HA induced adopted mainly a cable-like structure conformation, while in epithelial cells displayed pericellular location.

Discussion

The pattern of expression of HA, CD44, versican and TN-C in porcine ileum was altered in pathologic conditions such as porcine proliferative enteropathy and PMWS, which suggests that these molecules could be involved in the pathogenesis of these diseases. Changes in cellular HA expression were observed after treatment of cells with poly(I:C). In SMCs HA adopted a cable-like conformation, as has been already described in different human cell types (1). These structures are thought to play a role in inflammation through lymphocyte binding. Thus, they could also be involved in the mechanism of infectious intestinal diseases.

Acknowledgements

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P.022
DOES PCV2 REDUCE THE IMPACT OF SUBSEQUENT INFECTION WITH LAWSONIA INTRACELLULARIS?

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Introduction

Lawsonia intracellularis (Li) and Porcine Circovirus type 2 (PCV2) infections have both been associated with diarrhoea in weaned pigs and the two infections are often grossly indistinguishable. The prophylactic (vaccines) and therapeutic (antibiotics) strategies for the two infections are different; therefore it is of utmost importance to employ diagnostic tests to distinguish between them at the herd level. The exclusive detection of PCV2 and Li antibodies and/or DNA in serum or other samples, is not diagnostic for the two infections. Therefore, laborious, time-demanding and expensive histopathological examinations often on several animals are required. Thus, there is a need for more sophisticated discriminating diagnostic tests, which can be performed on samples from live animals. The aims of the study were to investigate the interaction between PCV2 and Li.

Material and methods

Briefly, 24 pigs free from PCV1 and PCV2, PRRSV, swine influenza virus (SIV), porcine respiratory coronavirus (PRCV), and a number of other microbiological agents, including *Mycoplasma* spp. and Li were divided into three groups each with 8 pigs. The treatments were as follows: pigs in group 1 were mock inoculated (PK15 cells) post infection day 0 (PID0) and mock inoculated (SPG buffer) PID14. The pigs in group 2 were mock inoculated (PK15 cells) PID0 and inoculated with Li PID14. Pigs in group 3 were inoculated with PCV2 PID0 and with Li PID14. The pigs were killed and necropsied on PID 50.

The PCV2 challenges were performed using a previously described procedure (1) with a few modifications: A Danish field isolate (PK67782-13 – passage 10 - titer 4.9 log10/Ml) of PCV2 genotype IIb were used as virus and the inocula were given by the nares and orally. The Li challenges were performed using the procedure previously described (2), using a tissue homogenate (titer 9-10 log/ML).

Clinical signs and body temperatures were recorded daily. The pigs were weighed weekly throughout the experimental period. Blood samples and faeces were collected prior to the first inoculation and at least once weekly from all animals. Tissue samples of tonsil, liver, spleen, kidney, lung, thymus, and lymph nodes (mesenteric, bronchial, prescapular and superficial inguinal), duodenum, ileum, caecum and colon were collected at necropsy for histopathological examination and immunohistochemistry.

The level of PCV2 and Li excretion were assessed by testing of all faeces samples by the use of quantitative real time PCRs (3,4). Similarly, the serum samples were tested for PCV2 in real time PCR. The serum samples were tested for antibodies against PCV2 and Li using ELISAs routinely performed at the institute.

Results

The laboratory tests performed on the inocula showed that the Li inoculum were contaminated with PCV2, thus the pigs in group 2 received PCV2 in addition to Li at PID14. No clinical signs were seen in pigs from group 1 (mock infected controls). All pigs in group 2 (challenged with Li at PID14) got severe, watery diarrhoea 4 days after challenge lasting 1-2 days and the pigs were clearly clinically affected and depressed. Interestingly, the pigs in group 3 (infected with PCV2 PID0 and challenged with Li PID14*)* only got a very light and temporal diarrhoea (not watery) 2 days after Li challenge and these pigs were not clinically affected. Tests of faeces samples from the pigs in groups 2 and 3 showed that the excretion of Li on PID 35 and 42 were one log10 (group means) lower in pigs challenged with PCV2 on PID0 and Li on PID14 compared to the pigs challenged with Li only.

Discussion

The differences in clinical signs and shedding between pigs in groups 2 and 3 may either indicate that previously exposure to PCV2 reduced the subsequent host response to Li or that the PCV2 exposure of the pigs at PID0 had a direct protective effect on the PCV2/Li exposure at PID14.

Acknowledgements

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P.023

INVESTIGATION OF PCV2-STATUS IN DANISH HERDS - ONE YEAR LONGITUDINAL STUDY

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Introduction

Based on the promising results of using real time PCR for the diagnosis of typical PMWS at the herd level, several diagnosticians now use PCV2 serum profiles to clarify whether a particular herd suffers from PCVD. Typically, the PCV2 copy number is measured in 5-10 blood samples from each of 3-5 different age groups of growing pigs (1). If the appearance of clinical signs of disease coincides with moderate to severe viraemia (>$10^{5\text{-}6}$ copies of PCV2/ml serum in a relevant number of samples), the PCVD diagnosis is considered supported.

In 2009, the diagnostic laboratory at the National Veterinary Institute (NVI) of Denmark completed PCV2 profiles from 196 herds, assumed to be sampled because of a suspicion of PCVD. In 28% of the herds, the level of PCV2 in serum was below the detection limit of the assay (<10^3 copies/ml) in all pigs. This was in contrast to the results of studies performed five to six years ago, which showed that the herd prevalence of PCV2 in Denmark was above 90%. Hence, the herd prevalence of PCV2 might have decreased, or the cross-sectional herd profiles might not always reveal the PCV2 infection due to fluctuation in the level of PCV2 circulation over time.

The objective of this study was to clarify, whether the negative PCV2 profiles mean that some herds, at this stage of the PCV2 “epidemic”, are able to clear the infection or whether the negative profiles are due to a temporal fluctuation in the PCV2 infection pressure at the herd level.

Materials and methods

Based on diagnostic submissions to NVI, eight finishing herds with a negative PCV2 profile and willingness to participate was selected for further investigations. The PCV2 profiles were repeated, and two herds with a negative profile at this 2nd testing were included in the study.

Herd 1 was a finishing herd buying 1000 PCV2-vaccinated (Ingelvac CircoFLEX®) pigs every 7th week. All pigs were born from sows vaccinated with Ingelvac CircoFLEX®. One batch of pigs (May) was not vaccinated. Herd 2 was a farrow-to-finish herd that do not vaccinate against PCV2.

The included herds were blood sampled every second month during at least one year, i.e. six samplings per year. At each sampling, blood samples were collected from each of 10 pigs at 30, 50 and 100 kg live weight. In herd 1, only 2 age groups of pigs were present at a time, therefore samples were taken from 45 and 100 kg pigs. This herd has been tested 8 times. The level of PCV2 was quantitated by testing serum samples using quantitative real time PCR at NVI (1).

Results

All samples taken in Herd 1, except samples from May, have been negative when tested for PCV2 in PCR. In May, samples from both Young and Old pigs were positive in PCR (table 1). In Herd 2, the first two samplings were negative and the last four were PCV2 positive in PCR.

Table 1: PCV2 virus load in serum samples from different age groups of pigs in herd 1 (PCV2 copies/ml)

	Young pigs (45 kg)	Old pigs (100 kg)
January	neg*	neg
March	neg	neg
May	10^4	10^7
June	neg	neg
August	neg	neg
October	neg	neg
November	neg	neg
January	neg	neg

*Negative: The level of PCV2 in serum was below the detection limit of the assay (<10^3copies/ml)

Discussion

The detection of PCV2 in herd 1 seen in May might be due to the missing PCV2 vaccination of this batch of pigs, but an acute PRRSV-US infection at the same time may also have influenced the PCV2 levels. After the reintroduction of the PCV2 vaccine, the PCV2 level was again below the detectable level in the PCR, although PRRSV continued to circulate in the herd.

The results from herd 2 suggested that the negative profile found in Danish herds may be explained by temporal fluctuation in the PCV2 level and not due to PCV2-free herds. However, as seen in herd 1, PCV2 vaccination of the piglets might prevent fluctuation in the PCV2 level when performed continuously, also during acute PRRSV-US infection.

Acknowledgements

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P.024
PCV2 VIROLOGICAL PROFILES IN PCVD AFFECTED AND NON-AFFECTED HERDS

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Introduction

Porcine circovirus type 2 (PCV2) is responsible for various symptoms that impair pig growth and are described as PCV2 diseases (PCVD). PCV2 shedding in faeces and the serological profile might be different in PCVD affected (AH) and non-affected herds (NAH) (1). Both the infection pressure in the environment and the serological profile of the herd might give an indication on the risk to suffer from PCVD and, by consequence, on the benefit from vaccination against PCV2. In addition, the relationship between antibody (Ab) titres in serum and the amount of virus shed by an infected animal remains unclear.

The aim of the study was to establish PCV2 virus load in faeces and Ab profiles of pigs from PCVD AH and NAH.

Materials and methods

A cross sectional study was conducted in 10 farrow-to-finish herds not vaccinating against PCV2 and with at least 180 sows (min. 180; max. 500). Poor performing herds in which PCVD was diagnosed from at least 1 out of 5 nursery piglets at necropsy, were selected as PCVD AH (n = 5). Herds without poor performance in the nursery and fattening pigs were selected as NAH (n = 5).

Blood was collected from pigs of 3 (in the farrowing unit), 9, 15, and 21 weeks of age (WOA) Five pens were randomly selected per group of age and, within each pen, 1 pig was sampled. Blood was also collected from 5 individual gilts and 15 sows within each herd. In the selected pens, three different fresh faeces samples from the floor were pooled to one sample. Individual faeces samples were collected from the floor from the selected gilts and sows. The serum of the blood samples and the faeces were frozen and shipped in dry ice to the laboratory. The serum was used for PCV2 Ab detection (SERELISA, Synbiotics, Lyon) and faeces were analyzed by qPCR (CODA laboratory, Brussels)

Possible differences between AH and NAH were compared using Kruskal-Wallis test (Ab titres in serum and virus titres in faeces) or using Chi-square test (proportion of qPCR positive pigs) Associations between Ab titres in serum and PCR titres in faeces were investigated using Spearman rank correlation.

Results

There were no significant differences regarding PCV2 Ab titres between the two types of herds. The average percentage of positive faeces samples by qPCR was higher (P< 0.05) in PCVD AH than in NAH for pigs of 9, 15 and 21 WOA (Table 1). The virus titre in faeces was higher (P<0.05) from pigs of 9 and 15 WOA and also in sows from PCVD AH (Table 1). The correlation between serum Ab titres and PCR titres in faeces was -0.151 (P= 0.248).

Table 1: Average percentage of faeces samples positive to PCV2 and their average PCV2 titres (Log 10 copies/gram) by qPCR from PCVD affected (n=5) and non-affected herds (n=5). For each parameter different superscript within a row indicate significant difference (P<0.05)

	Affected herds		Non-affected herds	
Age group	Positive PCR (%)	PCV2 Titres[1]	Positive PCR (%)	PCV2[1] Titres
3 w	29.2 ± 10.3	4.9 ± 0.7	16.0 ± 16.7	5.2 ± 0.4
9 w	76.0 ± 43.0^a	7.6 ± 1.7^a	25.0 ± 27.9^b	5.4 ± 0.2^b
15 w	95.0 ± 8.0^a	6.4 ± 1.1^a	48.0 ± 50.2^b	1.4 ± 6.5^b
21 w	76.0 ± 22.0^a	3.7 ± 4.9	32.0 ± 30.3^b	6.2 ± 0.7
Gilts	60.0 ± 20.0	4.9 ± 0.8	35.3 ± 33.25	5.4 ± 0.2
Sows	28.6 ± 33.0	5.2 ± 2.7^a	27.5 ± 22.9	4.4 ± 1.2^b

[1]PCV2 titres were measured only in the positive samples

Discussion

With the current available test and the limited population involved in this study, ELISA serology did not allow differentiating PCVD AH from NAH, and Ab titres were not associated with viral shedding in faeces. Although there was a high variation between the herds, qPCR testing in faeces clearly showed that virus shedding was higher in piglets of 9, 15 and 21 w of age in AH, which is in agreement with previous studies (1,2). Therefore, high quantities of PCV2 in fresh faeces in the pens could be an indicator of a problem herd. This study also showed PCV2 shedding at early ages which could indicate an early infection of the piglets either by transmission from sows or vertical transmission (2). Therefore, a good maternal immunity might be important to protect young piglets against clinical disease.

Acknowledgements

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P.025

WHY PCV2 VIREMIA MATTERS TO SWINE PRACTITIONERS

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Introduction

Viremia results in immune system activation that leads to a redirection of nutrients that may result in a decrease in ADG and/or an increase in feed-to-gain ratio (1). Subclinical infections with PCV2 are common (2,3). They are known to occur if vaccination does not prevent viral replication (4). These PCV2 subclinical infections do not require co-infections.

The objective of this paper is to describe the prevalence and production impact of viremia in pigs while comparing two commercial circovirus vaccines or a placebo under field conditions.

Materials and methods

A PRRS- and M. hyopneumoniae-free system was used for the study. At weaning, pigs were randomly assigned to be vaccinated with a one dose commercial PCV2 vaccine (n=1026) (1D); a two dose commercial PCV2 vaccine (n=1020) (2D); or saline as a placebo (n=100) (control). Both PCV2 vaccines were used according to manufacturer's label recommendations. Individual pig weights were collected when pigs were 3, 11 and 19 weeks of age and just prior to market at about 110 kg. Individual ADG was determined between each pair of weights.To determine extent of PCV2 viremia, blood was collected from a random sample of 122 pigs at 3, 9, 15, 19, and 23 weeks of age and during the final week of shipping to market. Serum was tested with a qPCR. The proportion of viremic pigs at each sampling point by treatment group was determined. Difference in proportions was determined with a chi-square test. ADG was compared by group and by viremia status using a multiple linear regression after controlling for weaning cohort and starting weight.

Results

Starting weights did not differ by group. More pigs in the 1D (47%) and control groups (85%) were viremic than in the 2D group (8%) ($p<0.01$). More control pigs (73%) were viremic on at least 2 samples than 2D pigs (0%) or 1D pigs (24%) ($p<0.05$). Pigs that were viremic more than once, tended to have a lower ADG (by 33.8 gm/d) than pigs that were never viremic ($p=0.11$). The proportion of viremic pigs remained at 3% to 4% for the entire grower-finisher phase for the 2D pigs. However, this proportion increased from 11% to 39% for the 1D pigs and from 53% to 63% for the control pigs from 15 to 19 weeks of age. The ADG in the 2D group was 42g / day higher than in the 1D group from 19 weeks to market age ($p<0.01$). The coefficient of variation of ADG in the 2D group (19%) was lower than that of the 1D group (28%) indicating that there was more variation in the growth of 1D group.

Discussion

The proportion of viremic pigs was higher in the control group than in the vaccinated pigs. The pigs receiving the 2D vaccine maintained a low level of viremia throughout the finisher phase whereas the pigs in the 1D group exhibited a rising proportion of viremia toward the end of the finishing phase. This viremia was associated with a lower ADG and more variation in growth performance, particularly during the final phase of production. This is the least efficient time of growth, feed conversion and facility use. This variation extends the time for marketing. Hence, viremia contributes to the overall cost of production in the 1D group.

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P.026

SIGNIFICANT PREDICTORS OF PCV2 VIREMIA IN AN UNVACCINATED CANADIAN FARM WITH MODERATE PORCINE CIRCOVIRAL DISEASE (PCVD)

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Introduction

A Canadian field study in a farm with moderate porcine circoviral disease (PCVD) affecting nursery pigs was performed with the objective of assessing relationships among porcine circovirus type 2 (PCV2) viral load, mortality, morbidity, and growth rate and a number of pig- and production-related risk factors. This report focuses on predictors of PCV2 viremia from birth to 15 weeks of age.

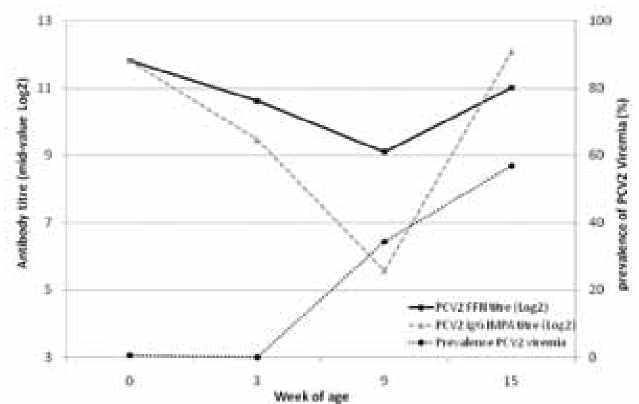
Materials and methods

160 piglets were monitored from birth to 15 weeks in a 600 sow farrow-to-finish research farm with moderately elevated post-weaning mortality (nursery 5.4±2.3%, finisher 4.6%±1.9%) attributable to PCVD. Two high and 2 low birth weight (BW) piglets per litter blocked by parity and farrowing week were weighed at birth, and 3, 9 and 15 weeks of age. Available stillborns from each dam were tested for parvovirus (PPV) and PCV2 by RT-PCR. Total IgG was measured in day 1 piglet sera. *PCV2 assessments:* Antibody (IMPA, FFN) and DNA (copies/ml) in serum were measured in blood collected from each sow at farrowing, and pigs at each time point. PCV2 DNA concentration was log (base 10) transformed and a dichotomous variable created to distinguish pigs that were or were not viremic at one or more time points. Antibody titres were converted to log (base 2) mid-value titres (1). *General immune competence* was assessed in heparinized blood collected at each time point using the Disease Resistance Assay for Animals (DRAA, Metadis Inc., Ottawa), a multihit assay that measures proliferative responses of PBMCs following stimulation with a panel of 5 mitogens, then aligns a pig's individual responses along 3 specific immune pathways (TCR/CD3-dependent, JAK/STAT, PKC-dependent) using Principle Component Analysis (PCA) (2). *Room temperature and relative humidity* were measured every 60 sec. for 42 days starting the day of weaning. *Statistical analysis*: Multilevel mixed-effects logistical regression (XTMELOGIT) was used to assess the predictors of PCV2 viremia at any time. Litter was included as a random intercept. Biologically plausible predictors were considered in the full model if $P < 0.2$. Significant variables were retained if $P<.05$ following backwards elimination.

Results

One pig was viremic when first tested at 18-24 hours after birth. No pig had detectable PCV2 DNA in serum at week 3 (Figure 1) indicating the absence of widespread vertical transmission. Forty-nine pigs (30.6%) had no detectable PCV2 DNA in serum at any time points. In the remaining majority (n=116, 68.4%), the average $\log_{10}$ PCV2 DNA levels in serum ranged from 0.9 to 3.3 copies/ml. Ten biologically relevant variables were unconditionally associated with the risk of a pig becoming viremic. Five of these were production-related factors (parity of sow, gender of pig, BW, BW category (high/low), average nursery temperature) and five were health attributes (total IgG day1, FFN antibody titre in sow, PCV2, PPV and FFN titres in piglets at week 0). Only two were significantly associated with a pig developing viremia and these variables were moderately correlated ($r_s = 0.53$). For each 2 fold increase in FFN antibody titre in sows at farrowing (i.e. 80 to 160), the odds of viremia occurring at any time point decreased 3.7 times. For each 3 fold increase in PCV2 IgG (IMPA) titre in piglets at day 1 (i.e. 50 to 150), the odds of viremia occurring at any time point decreased 2.3 times.

Figure 1. Mean PCV2 antibody and DNA prevalence in serum



Discussion

In this unvaccinated farm, the prevention of viremia at 9 and 15 weeks of age was entirely dependent on the presence of high PCV2 FFN antibody in sows at farrowing and PCV2 IgG in day 1 piglets.

Acknowledgements

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P.027

PREDICTABILITY OF PCV2 ANTIBODY LEVELS OF SOWS AND 3 WEEK OLD PIGLETS BASED ON A PREVIOUSLY PERFORMED HERD SCREENING

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Introduction

PCV2 piglet vaccination has become a global routine over the last years. Since the beginning of vaccine use, it has been discussed if vaccines work effectively in the presence of high levels of maternal derived antibodies (MDA). It has been suggested to perform a PCV2 antibody profiling of a representative number of piglets before vaccine implementation, to estimate proper vaccination timing (1). To know if this approach can be used under field conditions, it is important to gain knowledge about the variability of the antibody titers within a farm. If there is a good correlation between the antibody levels of all animals within a farm, then it might be possible to determine the antibody level status of the farm by means of a herd screening and adapt the vaccination protocol accordingly.

Materials and methods

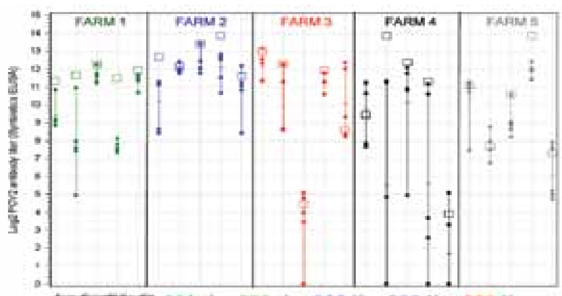
In total, 5 commercial Belgian farms participated in the study. In each farm, blood samples of 5 lactating sows were collected, 1 primipareous and 4 multipareous sows. In none of the farms sow vaccination against PCV2 was applied. Of each of the sows, 5 piglets were blood sampled. The piglets were randomly selected within each litter, but cross-fostered piglets were excluded. Piglets were unvaccinated against PCV2 at the time of blood sampling. All samples were taken 3 weeks post-farrowing.

The serum samples were analysed for PCV2 antibodies with the Serelisa® PCV2 Ab Mono Blocking ELISA (Synbiotics Corp). The results are expressed in Log 2 titers and samples with an antibody level below the detection limit are shown as zero.

Results

Figure 1 shows the variability in the antibody levels of all sampled animals. The squares indicate the antibody titre of a sow, the dots indicate the titre of a piglet. The dots (piglets) and the square (sow) on the same vertical line belong to the same litter and all the animals belonging to one farm have the same colour.

Figure 1 PCV2 antibody levels of sows and piglets in 5 commercial farms



Discussion

The results show a high variability of the PCV2 antibody levels between farms, sows and piglets within a farm and even between piglets within the same litter. The latter might be due to differences in colostrum uptake between piglets in a litter.

Due to the high variability in the antibody titers, it is impossible to predict the PCV2 antibody level of an individual piglet by means of a previously performed herd screening or even by measuring the antibody titer of the piglets' mother. Only sampling each individual animal can give accurate information on the antibody status of the animal. Herd profiling does not seem to be a useful tool to estimate the proper time of vaccination. Furthermore, depending on the vaccine used, it is not necessary, as it has been shown that a single dose of vaccine can break through high levels of MDA (2).

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P.028

PORCINE CIRCOVIRUS TYPE 2 LOAD IN SERUM IS NEGATIVELY CORRELATED WITH WEIGHT EVOLUTION IN POSTWEANING MULTISYSTEMIC WASTING SYNDROME AFFECTED FARMS

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Introduction

Porcine circovirus type 2 (PCV2) is associated to different conditions collectively named porcine circovirus diseases (PCVD). Postweaning multisystemic wasting syndrome (PMWS) is considered the most economically significant one. PMWS clinical course is characterized by a variable proportion of wasted pigs and high lethality.

Available PCV2 vaccines have demonstrated satisfactory results by reducing PCV2 load and prevalence and ameliorating PMWS clinical signs by improving growth performance and producing more homogeneous pig batches at slaughter (1). Therefore, it seems that levels of PCV2 viremia may affect growth performance. Based on such background, the aim of the present study was to assess the existence of a correlation between viral load in blood and growth evolution in pigs from PMWS affected farms.

Materials and methods

Data from 160 pigs included in a larger study of 1062 pigs (2) were used. These piglets came from two almost identical 5000-sow multisite farms (A, n=86; and B, n=82) separated by 300 m. Both farms had previously diagnosed as PMWS. Weaned pigs coming from each sow farm were placed in the same nursery farm but in different rooms. Seven weeks after weaning, all animals were placed in the same fattening farm and room, but placed in different pens.

Pigs were weighted and bled at 3, 9, 15 and 21 weeks of age. Standard PCV2 PCR (3) was performed in all blood samples, and those that tested positive were also analysed by means of real time quantitative PCR (qPCR) (4). Area under the curve of qPCR (AUCqPCR) and weight (AUCW) was assessed for each pig.

The 160 piglets were selected following the criterion that they had complete information on weight, PCV2 PCR and qPCR during the whole trial period; thus, these pigs survived during the complete study period. Furthermore, piglets in the percentile 5 of AUCW were excluded from the study to avoid animals with clinical PMWS that survived the disease. Therefore, studied animals could apparently represent pigs with PCV2 subclinical infection.

A Spearman's Rank correlation analysis was performed between AUCqPCR and AUCW.

Results

A PMWS outbreak was observed between 9 and 15 weeks of age in the studied population (n=1062), with 6.5% mortality (66.9% of it was diagnosed as PMWS) (data not shown). Weight evolution, proportion of PCV2 viraemic pigs and their viral load dynamics are shown in Table 1. Viral load dynamics was negatively correlated with weight evolution (ρ=-0.203; p=0.010). Thus, the higher the AUCqPCR during the postweaning period, the lower AUCW.

Table 1. Percentage of PCV2 viraemic pigs (PCR),mean (±SD) $\log_{10}$ PCV2 viral load (qPCR) of PCR positive pigs and mean (±SD) weight dynamics during the study.

Weeks of age	% PCV2 PCR positive	PCV2 qPCR (log10 genome copies/ml)	Weight (kg)
3	0	-	6.5±1.4
9	50	5.2±5.7	22.5±3.5
15	80	3.7±4.2	51.9±7.7
21	60	3.2±3.6	82.5±11.8

Discussion

The present study shows that growth performance was negatively and significantly influenced by the PCV2 load in serum. This finding agrees with the improvement of growth performance in PMWS affected farms after PCV2 vaccination (1). Taking into account that these 160 pigs were apparently examples of PCV2 subclinical infection, it is not surprising that the positive effects of PCV2 vaccination also apply to subclinical infections (1).

Acknowledgements

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P.029

PRELIMINARY RESULTS ON THE RELATIONSHIP BETWEEN SOW AND THEIR LITTER AVERAGE PCV2 ANTIBODY TITRES AS AN INDICATOR OF COLOSTRUM INTAKE

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Introduction

PCV2 is recognized as the etiologic agent of PMWS (1) and is responsible for severe economic damages in porcine herd worldwide.

A low level of maternally derived immunity has extensively been shown as a risk factor for the onset of clinical PCVD in pigs (2, 3). The objective of this preliminary study was to evaluate on a low number of farms whether the correlation between dam and piglet PCV2 antibody titres could be a good indicator of the quality of colostrum intake under Italian conditions.

Materials and Methods

Four non-PCV2-vaccinated sow farms (A, B, C, D) were selected based on their history of PCVD: in all herds had experiencing clinical signs characterised by wasting, respiratory signs, and growth retardations were mainly associated with an increase in the mortality rate (4). In each farm, 9 to 12 sows and for each sow, 4 of their piglets were randomly selected and blood sampled 20 days after farrowing. Sera were submitted to a semi-quantitative ELISA test (IZLER Lodi, Italia) expressed at the limit of the class in titres (0, 10, 100, 1000, 10000). Geometric mean antibody titres of the 4 piglets per litter were plotted against the titre of their dam.

Although sows and piglets, geometric mean PCV2 antibodies titres were expressed in log 10, and correlation coefficient for each farm was determined.

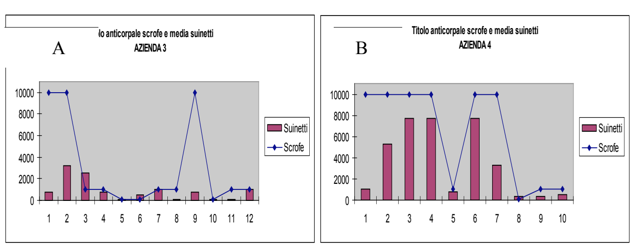
Table 1: Gross characteristics of the farrowing management

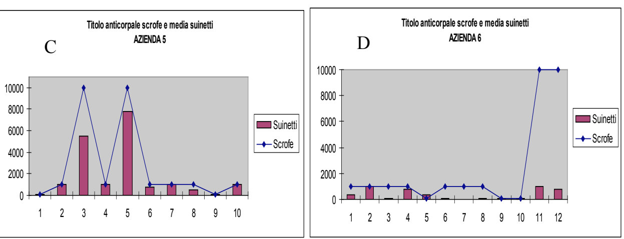
Farm	A	B	C	D
Induced farrowing		x		
Synchronisation	x			
Assisted farrowing	x	x	x	
Adoption within 24h		x	x	x
Mix at weaning	x	x	x	x

Results

Figure 1 shows PCV2 antibodies titres from sows and piglets in the 4 farms. The calculated R^2 values are 0.376, 0.734, 0.851 and 0.170, for farms A, B, C & D, respectively.

Figure 1: (A, B, C, D) individual antibody titres of sows and average titres of piglets





Discussion

The preliminary results obtained on a limited number of farms and animals shows that levels of antibodies are highly variable from sow to sow as well as from farm to farm. Conversely to farms A & D, there is a rather tight relation between sow and average piglet PCV2 antibody titres in farms B & C.

The correlation is clearly shown in farm C (p=0.00). However, this cannot be related to any of the gross characteristics of the farrowing management. Despite it has not been possible to relate the clear cut serological profiles of the farms to the management characteristics, it would be interesting to look more in depth to the potential clinical PCVD risk factors using the R^2 coefficient between sow and their litter average PCV2 log10 titres as an indicator of colostrum intake. Furthermore, this would potentially be used as a good routine technique to evaluate colostrum management in farms, on a more general purpose.

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P.030

EVOLUTION OF PORCINE CIRCOVIRUS TYPE 2 GENOME IN SPRAY DRIED PORCINE PLASMA SAMPLES TAKEN FROM 2009 TO 2010 IN SPAIN

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Introduction

Spray dried porcine plasma (SDPP) is a dehydrated product obtained from blood of healthy pigs at slaughterhouse. SDPP has many excellent properties as a feed additive that promotes the adaptation of the gastrointestinal mucosa to dietary changes at weaning (1), and significantly improves average daily gain (ADG), average daily feed intake (ADFI) and reduces diarrhoea scores (2,3). In the processing plant, the plasma is separated from red blood cells by centrifugation, concentrated and spray-dried at high temperature. The product is submitted to a thermal process of >80ºC throughout its substance and therefore commercially manufactured SDPP is a safe product even when highly resistant viruses such as PCV2 are considered (4, 5).

PCV2 vaccines have been shown to be very efficacious in controlling PCV2-related diseases such as postweaning multisystemic wasting syndrome (PMWS), and even PCV2 subclinical infections (6). In fact, they are able to decrease not only the prevalence of infection but also the viral load in serum of pigs (6). Therefore, the objective of this study was to monitor the PCV2 genome load in different batches of SDPP since 2009 to date.

Materials and methods

A total of 332 samples of SDPP from daily batches were monthly collected during a 14 month period (November 2009 to December 2010) at the Spanish SDPP producer plant. Samples came from 25 slaughterhouses which represent >60% of total Spanish pig production. The SDPP daily batches contained blood from roughly 50,000 animals sourced from different farms around Spain, primarily from (North-Eastern Spain). SDPP samples were resuspended (0.9%) and analysed by a previously described quantitative PCR (qPCR) to detect PCV2 (7). Each sample was analyzed by triplicate.

Results

PCV2 qPCR results of collected Spanish SDPP samples are represented as monthly means in Fig. 1. The overall mean of $\log_{10}$/ml PCV2 DNA copies in the 332 SDPP samples was 4.23±0.95. The decrease in PCV2 viral load in SDPP was significant over time ($p<0.001$).

Discussion

The present results indicate a progressive and significant decrease of PCV2 genome in SDPP samples over a period of 14 months in Spain. This scenario could be partially explained by the fact that during 2009 and 2010 the use of commercial vaccines against PCV2 has been increasing in the Spanish pig production. These vaccines have high degree of efficacy in terms of clinical protection and viremia reduction (6). Increased vaccine use may have had an effect on PCV2 genome copies reduction in SDPP lots by reduction of the number of chronic, non-clinically viremic animals at slaughter time.

It is noteworthy that when liquid plasma samples representing a much lower number of pigs (around 5,000 pigs) were taken, some PCV2 qPCR negative results were found in 8 of 16 samples (data not shown). Such results may suggest that an increasing number of finishing pigs are becoming non-PVC2 viremic at the time of slaughter.

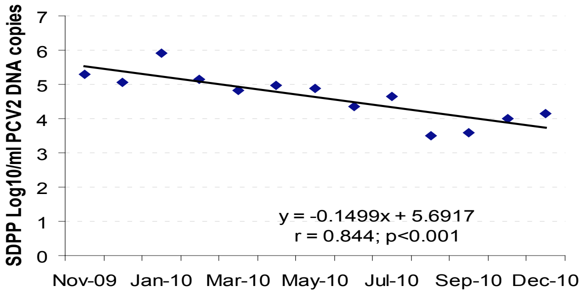

Fig 1. Monthly average (mean values of approximately 25 SDPP samples each) of $\log_{10}$/ml PCV2 DNA copies performed by qPCR)

Acknowledgements

Authors thank J.S. Villar for collecting all SDPP samples and E. Huerta, N. Navarro, E. Cano and L. Osaez for laboratorial and technical assistance.

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P.031

EVOLUTION OF ANTIBODY TITRES AGAINST PORCINE CIRCOVIRUS TYPE 2 IN SPRAY DRIED PORCINE PLASMA SAMPLES FROM 2009 TO 2010 IN SPAIN

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Introduction

Spray dried porcine plasma (SDPP) is a dehydrated product obtained from blood of healthy pigs at slaughterhouse. SDPP as a protein source significantly improves average daily gain, average daily feed intake, reduces diarrhoea scores (1,2) and promotes the adaptation of the gastrointestinal mucosa to dietary changes at weaning (3). Due to the special manufacturing process involved, commercially available SDPP is a safe product even when highly resistant viruses such as PCV2 are considered (4,5). Since most of the pathogens infecting the pig during its productive life give an antibody response which is still detectable at slaughter age, SDPP is a priori a good sample to monitor evolution of antibodies in pig populations, especially in regards enzootic agents.

Therefore, the objective of this study was to monitor the antibody titres against PCV2 in different batches of SDPP since 2009 to date.

Materials and methods

A total of 278 samples of SDPP from daily batches were monthly collected during a 14 month period (November 2009 to December 2010) at a Spanish SDPP producer plant. Samples came from 25 slaughterhouses which represented >60% of total Spanish pig production. The SDPP daily batches contained blood from roughly 50,000 animals sourced from different farms around Spain, primarily from the North-Eastern part of the country.

SDPP samples were re-suspended (0.9%) in PBS and tested by an immunoperoxidase monolayer assay (IPMA) to detect PCV2 antibody titres (6).

Results

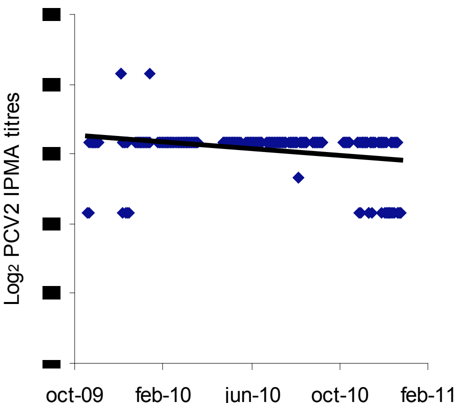
IPMA antibodies against PCV2 in collected Spanish SDPP samples are represented as individual values in Figure 1. The overall $\log_2$ mean of the 278 samples was 14.2±0.64. The decrease in PCV2 antibody titres was significant over time ($p<0.001$).

Discussion

Results of the present study showed that PCV2 antibody titres in SDPP samples in Spain showed a slow decreasing tendency over a period of 14 months. These observations coincide with a statistically significant reduction of PCV2 viral load in the same SDPP samples (7).

The use of commercial vaccines against PCV2 has been increasing in the Spanish pig production during 2009 and 2010. These vaccines have high degree of efficacy in terms of clinical protection and viremia reduction (8). The increased vaccine use seems to have had an effect on reduction of the number of non-clinically viremic animals and also in the amount of total antibodies. Since proportion of PCV2 infection among vaccinated pigs is significantly lower than non-vaccinated ones, it was also expected that amount of antibodies in a population of vaccinated pigs would be lower than a population of non-vaccinated ones, as it has been described in PCV2 vaccine efficacy studies (9).

Figure 1. Values of $\log_2$ PCV2 IPMA titres of individual SDPP batches ($y = -0{,}0016x + 76{,}932$; $r= 0{,}302$ $P<0{,}001$).



Acknowledgements

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P.032

DIRECT AND INDIRECT EFFECTS OF VIRAL CO-INFECTIONS AND FARM MANAGEMENT ON POSTWEANING MORTALITY IN PIG FARMS: A PARTIAL LEAST SQUARE – PATH MODELING APPROACH

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Introduction

Pigs are commonly infected by a number of viruses during the production period. Some of these viruses are sufficient to produce disease whereas others, such as porcine circovirus type 2 (PCV2), require the presence of other infectious/non-infectious triggers (1). Thus, viral infections are linked to postweaning mortality (PM) in pig farms. However, PM has been also related to farm management conditions during both nursery and fattening periods. Since both pathogens and farm management practices affect PM, an integrative statistical approach would be required to understand their direct and indirect effects.

In this work, it was explored whether combined direct and indirect effects of porcine circovirus type 2 (PCV2), various common concurrent viruses (porcine reproductive and respiratory syndrome virus, PRRSV; swine influenza virus, SIV; and Aujeszky' s disease virus, ADV), and some common management practices influenced nursery and fattening mortalities.

Materials and methods

To assess seroprevalences to PRRSV, SIV, ADV and PCV2, fifteen 20 week-old pig sera were collected from 42 indoor pig farms located in North-Eastern Spain. Management conditions that may modulate PM in nursery and fattening pigs were also recorded in each farm. Specifically, data on animal flow, age at weaning, age entering fattening units, pig density and presence/absence of solid partitions between pens were recorded.

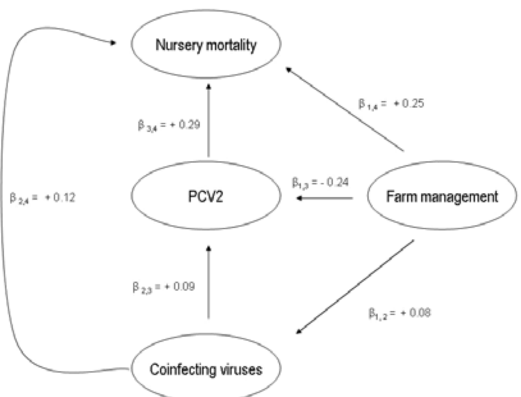
To find causal relationships between selected variables, a partial least square path modelling (PLS–PM) approach using the plspm package (2) of the R version 2.12.1 (R Developmental Core Team) was used. Path (β) and correlation (r) coefficients were validated by bootstrapping.

Results

Nursery period (Fig. 1). Continuous animal flow (r=0.23) and elevated pig densities (r=0.19) directly increased pig nursery mortality. Also, a higher age at weaning was associated with a reduction of nursery mortality, mainly by decreasing PCV2 prevalence (r=-0.23). Curiously, co-infecting viruses had a minor direct influence on nursery mortality ($\beta_{2,4}$=0.12) but an important indirect effect throught PCV2 ($\beta_{2,3}$+$\beta_{3,4}$=0.38).

Fattening period. Higher ages at fattening entry were directly associated with increased PCV2 (r=0.28) and co-infecting viruses prevalences (r=0.31). Furthermore, PCV2 ($\beta_{3,4}$=0.18, r=0.16) and co-infecting viruses ($\beta_{2,4}$=0.29 for direct and $\beta_{2,3+}\beta_{3,4}$=0.29 for indirect effects, r=0.31) prevalences were both positively related to fattening mortality.

Figure 1. Path diagram representing both direct and indirect influences of farm management, prevalence of some co-infecting viruses and PCV2 on nursery mortality in 42 Spanish pig farms.



Discussion

The present results revealed that farm management exerted direct and indirect effects on PM. The magnitude of these effects was different depending on the production period, with a main role in the nursery. The direct and indirect effects of PCV2 and co-infecting viruses on PM were always positive (increased PM) in both periods, with main impact during fattening period. Moreover, higher nursery mortality was observed when prevalences of PCV2 and co-infecting viruses were high.

To the authors' knowledge, this is the first time that a PLS–PM approach has been used to measure the relationships among farm management and prevalence to different viruses on PM in pig farms.

Acknowledgements

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P.033

COMPARISON OF FOUR DIFFERENT QUALITATIVE SEROLOGICAL METHODS FOR THE DETECTION OF ANTIBODIES AGAINST PCV2

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Introduction

Antibodies against PCV2 can be detected in most pigs under field conditions, due to the ubiquitous character of the virus (1). For herd profiling a broad selection of various test systems is offered by different laboratories (2). The aim of the present study was to compare four serological test systems with regard to their ability to detect antibodies in piglets vaccinated against PCV2 at different ages.

Materials and methods

In total, 126 piglets were included and randomly allocated to two different groups, which received a single dose of 1 ml Ingelvac CircoFLEX® (Boehringer Ingelheim Vetmedica, Ingelheim, Germany) either at 14 or 28 days of age. Blood samples were regularly collected from allocation to slaughter as described by Viehmann et al. (3). All samples were tested for the presence of anti-PCV2 antibodies by the Ingezim® Circovirus IgG/IgM (Ingenasa, Spain, data only shown for IgG), the Ingezim® Circo IgG ELISA (Ingenasa, Spain), the Serelisa® PCV2 Ab Mono Blocking (Synbiotics Europe SAS, France) and by the Indirect Fluorescence Antibody Titration (IFAT; BioScreen GmbH, Muenster, Germany). The results from all different tests were evaluated qualitatively. Sera that gave an inconclusive result by the Ingezim® Circo IgG ELISA were considered positive.

Results

The percentage of sera tested positive for IgG by the Ingezim® Circovirus IgG/IgM decreased over time, with no samples tested positive after 12 weeks of age. Almost all serum samples remained positive by the other test systems throughout the trial (Fig. 1). No increase of IgM could be detected by the Ingezim® Circovirus IgG/IgM over the whole period (data not shown). The Ingezim® Circo IgG ELISA, the Serelisa® and IFAT gave mostly congruent results, while the Ingezim® Circovirus IgG/IgM showed the lowest number of congruent results. The results from the different test systems are shown in Table 1. No significant differences could be detected between the two treatment groups in any of the tests.

Discussion

All used tests systems were able to detect anti-PCV2 antibodies in swine sera. In general, the Ingezim® Circovirus IgG/IgM ELISA seemed to be less sensitive in detecting antibodies against PCV2 than the Ingezim® Circo IgG, the Serelisa® test or the IFAT. However, it is not well defined what kind of antibodies are detected by the different test systems. This should be kept in mind when comparing and interpreting test results within and between laboratories (2). No differences were detected in the number of positive sera between the pigs vaccinated at 14 days or 28 days of age.

Figure 1: Percentage of animals tested positive by the four test systems

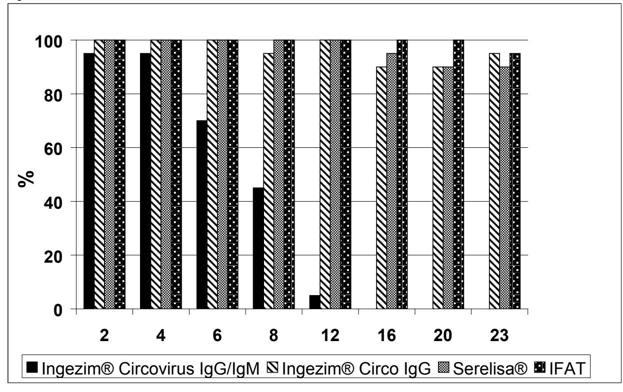

Table 1: Number of positive (+) and negative (-) sera by each test system

Assay		Ingezim® Circo IgG		Serelisa®		IFAT	
		-	+	-	+	-	+
Ingezim® Circovirus IgG/IgM	-	6	92	5	93	1	97
	+	0	62	0	62	0	62
IFAT	-	0	1	0	1	-	-
	+	6	153	5	154	-	-
Serelisa®	-	0	5	-	-	-	-
	+	6	149	-	-	-	-

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P.034
QUANTITATIVE ANALYSES OF ANTIBODIES AGAINST PCV2 BY THREE DIFFERENT SEROLOGICAL METHODS

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Introduction

To prevent PCVAD and associated economical losses, several PCV2 vaccines have been efficiently used over the last years (1). For herd profiling a broad selection of various test systems is offered by different laboratories (2). At present, routinely used serological diagnostic tests are not able to evaluate the efficacy of the vaccination (3). In the present study four serological test systems were analyzed with regard to their ability to quantify antibodies in piglets vaccinated against PCV2 at different ages.

Materials and methods

In total, 126 piglets from a 65 sow farrow-to-finish farm were randomly allocated to two different groups, which were either vaccinated against PCV2 (Ingelvac CircoFLEX®, Boehringer Ingelheim Vetmedica) at 14 or 28 days of age. Blood samples from 20 pigs were regularly collected from inclusion to slaughter as described by Viehmann et al. (4). All samples were analyzed by the Ingezim® Circo IgG ELISA (Ingenasa, Spain), the Serelisa® PCV2 Ab Mono Blocking (Synbiotics Europe SAS, France) and by the Indirect Fluorescence Antibody Titration (IFAT; BioScreen GmbH, Muenster, Germany).

Results

A decrease in antibody titers was observed in all three quantitative tests between the 2nd and 23rd week of life (Fig.1). In general, the Spearman´s coefficient of correlation was highly significant for the antibody levels measured by the three test systems (p=0.001). Nevertheless, differences of more than 8 log2 levels could be detected for individual serum samples between the three test systems (Fig.2) By analyzing the Serelisa® results, an increase in antibody titers was observed from week 16 to 20 in 3/10 pigs vaccinated at day 14 and 2/10 pigs vaccinated at day 28. Serum samples, that were collected from those animals in weeks 12, 16 and 20, were tested by qPCR for the presence of PCV2 to exclude an infection. All samples yielded a negative result by qPCR.

Discussion

In general, antibody titers from the three quantitative test systems showed a good agreement. However, a certain degree of variation could be detected for individual samples in the different tests. It has to be kept in mind, that different types of antibodies are measured by the different test systems.

The increase of antibody titers measured by the Serelisa® could not be related to positive findings in qPCR. Therefore, antibody levels have to be interpreted with care (2). No increase in antibody titers could be observed after vaccination in both groups. This is in line with other studies, where PCV2 vaccination did not induce seroconversion, but pigs showed a good protection against PCVAD (3). Hence, serological analysis is not suitable to evaluate the efficacy of vaccination against PCV2.

Fig. 1: Mean antibody titers obtained by the quantitative test systems

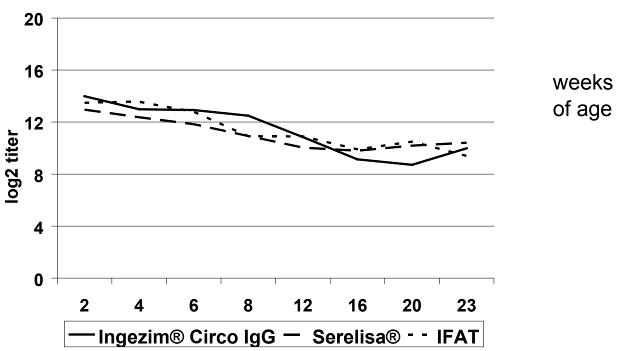
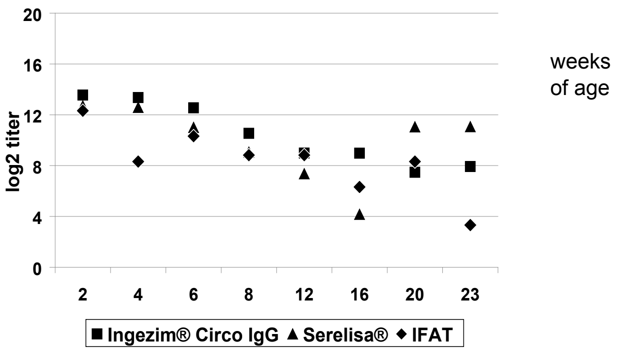


Fig. 2: Antibody titers from one serum sample obtained by the three test systems



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P.035

MEASUREMENT OF PORCINE CIRCOVIRUS TYPE 2 (PCV-2) ANTIBODY TITER IN PIG SERUM USING SERELISA® PCV-2

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Introduction

Porcine circovirus type 2 (PCV-2) is a small DNA virus that is widespread among swine herds world wide. The diagnosis of PCV-2 disease is complex and is based on detection of the virus and the presence of microscopic lesions and clinical signs consistent with the disease.

The main serological test currently in use is an indirect immunofluorescence antibody (IFA) test, which is not standardized between the laboratories and produces varying results from lab to lab. The Synbiotics SERELISA® PCV-2 Ab Blocking ELISA test uses a blocking immunoenzymatic technique for the measurement of anti-PCV-2 antibodies in swine serum. It is a rapid, specific and sensitive immunoassay for both qualitative and quantitative analysis.

The objective of this study was to evaluate SERELISA® PCV-2 for measuring maternally-derived antibody (MDA) in piglets and antibody response after vaccination or field challenge.

Materials and methods

Study 1: Detecting antibody after vaccination and field challenge: Thirty-two piglets born from unvaccinated sow were vaccinated at 3 and 6 weeks of age with Circumvent® PCV (Intervet/Schering Plough). Six control piglets were not vaccinated. Serum samples were taken at 3, 6, 9, 14 and 25 weeks of age and tested by both SERELISA® PCV-2 and IFA.

Study 2: Detecting maternal decay and antibody response to vaccination: Groups of 3 piglets born from vaccinated or unvaccinated sows were vaccinated with Circumvent®PCV at 3 and 5 weeks of age or 5 and 7 weeks of age. Groups of 3 piglets with or without MDA were not vaccinated. Serum samples were taken at 1,3,5,7,9,14 and 26 weeks of age and tested by both SERELISA® PCV-2 Ab Blocking ELISA and IFA.

Results

Study 1: The result showed similar trending for IFA and SERELISA® PCV-2 for both the vaccinated and control groups. However, SERELISA® PCV-2 is more sensitive than IFA. For vaccinated piglets, SERELISA® PCV-2 started to detect elevated antibody response after first vaccination at 6 weeks of age, whereas IFA only detects antibody at 9 weeks of age after booster vaccination. SERELISA® PCV-2 results showed more linear response for detecting the antibody elicited by the vaccine than IFA. In addition, SERELISA® PCV-2 testing indicated the active immunity lasted until 14 weeks of age, but IFA result showed that the antibody titer started to drop after 9 weeks. In unvaccinated piglets, both tests detected the field challenged response at 25 weeks of age.

Study 2: SERELISA® PCV-2 showed a more linear decline in MDA compared to IFA. Piglets with MDA vaccinated at 5 and 7 weeks of age exhibited a higher antibody titer compared to piglets vaccinated at 3/5 weeks as measured by SERELISA® PCV-2. IFA only detected a slightly increased antibody titer for 5/7 weeks vaccine schedule compared to the 3/5 weeks schedule. As expected, in the piglets with no MDA, vaccine schedule of 3/5 weeks provide earlier antibody response than the 5/7 weeks schedule. Therefore, SERELISA® PCV-2 may help to determine the optimum vaccination timing in the piglets with MDA.

Discussion

Compared to IFA, SERELISA® PCV-2 Ab Blocking ELISA is linear for detecting MDA or vaccine response to PCV-2.The test also detected higher antibody titer in vaccinated piglets and showed that the ELISA antibody response lasted longer, which proved excellent sensitivity. Our result also indicated that SERELISA® PCV-2 Ab Blocking ELISA measures the declining MDA and maybe useful for determining the timing of vaccination in piglets. The better vaccine response at 5/7 week scheme than 3/5 weeks is possibly due to MDA is higher at 3 to 5 weeks than 5 to 7 weeks; the high level of MDA may interfere the vaccine efficacy. However the impact on vaccine efficacy related to field challenge is unknown.

The results from field samples indicated that SERELISA® PCV-2 Ab Blocking ELISA effectively measures MDA in piglets and the antibody response after vaccination or field challenge. The PCV-2 antibody titers were consistent with the vaccination status and age of the pigs. ELISA is sensitive, specific and consistent for detecting the antibody to PCV-2 than IFA. Therefore, it is an efficient method for measuring antibody to PCV-2.

P.036

CORRELATION BETWEEN TWO SEROLOGY TECHNIQUES FOR ANALYSIS OF SPECIFIC ANTIBODIES AGAINST PCV2, ELISA (OD RATIOS) AND A TOTAL ANTIBODY ELISA

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Introduction

Porcine circovirosis is a worldwide disease caused by Type 2 Porcine Circovirus (PCV2). Serological techniques have been widely used to monitor the dynamics of PCV2 infection on farms affected and unaffected by PMWS (1). Specific tools are essential in order to determine the prevalence of the infection as well as the way in which PMWS develops (2). Adequate monitoring of the PCV2 antibody levels allows an understanding of the dynamics of the disease, the appropriate choice of vaccination program (3), and the evaluation of maternal immunity.

In Spain, quantitative antibody titration is not yet routine, but there is a kit available which measures IgG-IgM using Optical Density measurements. The purpose of this study was to discover if a correlation existed between the results obtained in the OD ratio ELISA, currently in frequent use, and an in-house ELISA designed by ISPAH, based on total antibody titration.

Materials and methods

A study was carried out on 90 animals on various farms in the north of Spain which were all serologically PCV2+ve and exhibiting clinical signs of late-stage disease. The animals were individually identified, and at three weeks of age were randomly assigned to groups which were treated with various vaccines against PCV2 or left unvaccinated to act as controls. Blood samples were taken at three weeks old and thereafter at four-weekly intervals until the pigs were sent for slaughter.

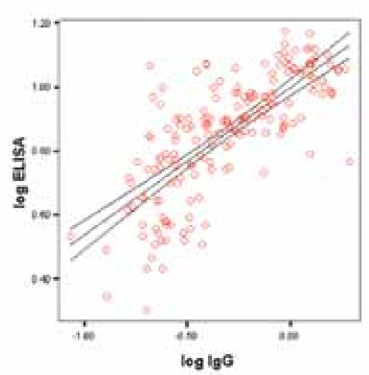
The serum was divided into two aliquots and stored frozen until the end of the study. All samples were then analyzed for PCV2 specific antibodies using the two kits under study: Ingezim ® from Ingenasa, an enzymatic capture immune-assay based on the use of three monoclonal antibodies and a recombinant antigen, in which PCV2 specific IgG and IgM are detected differentially, and an in-house PCV2 antibody ELISA developed by ISPAH R&D.

The results of the in-house ELISA, expressed as log_2 titres, range from 2 to 16 log_2; a titre of 5 log_2 represents a protective level, i.e. pigs with titres in excess of 5 log_2 pigs will be protected (4). The results obtained were subjected to regression analysis, applying a logarithmic transformation using SPSS 15.0 for Windows).

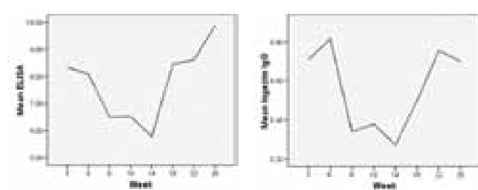
Results

A linear relationship exists between the dependent and the independent or predictor variables. The linear regression model is significant (F=112.351**, p<0.001). The independent variable, log IgG, has genuine value as a predictor (t=14.882**, p<0.001), which is not apparent with respect to the log IgM which was thus discarded in the model. The decimal logarithm of the ELISA totals can be predicted utilizing the formula: (equation of the regression curve): ELISA decimal logarithm = 1+ (0.46 x decimal logarithm of IgG). The correlation between the in-house ELISA titres and the O.D. ratio was determined by regression analysis between the ELISA O.D. and log_2 of the antibody concentration.

Graph 1: Graphical representation of the regression curve.



Graph 2: Average weekly values for total antibody and IgG



Discussion

A clear correlation exists between the results obtained from the two diagnostic kits studied. This enables the assessment of IgG in isolation, and the prediction of the levels of PCV2 antibodies in serum which, in turn, allows the extrapolation of the values obtained by the Ingezim® IgG-IgM kit (used routinely in Spain) to values for total antibody levels, and thus the measurement of immunity levels in samples from the field. This is of considerable importance for the control of porcine circovirosis, and means that the planning of prophylactic regimes can be optimized.

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P.037

COMPARISON OF AN IMMUNOPEROXIDASE MONOLAYER ASSAY AND A BLOCKING ELISA TEST TO DETECT ANTIBODIES AGAINST PORCINE CIRCOVIRUS TYPE 2

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Introduction

Porcine circovirus type 2 (PCV2) infection is associated to several swine diseases collectively referred as porcine circovirus diseases (PCVD) (1). Serological studies at the farm level are essential to monitor the viral infection as well as vaccination. Moreover, antibody detection can be useful to evaluate the optimal timing of vaccination. Immunoperoxidase monolayer assay (IPMA) is one of the most useful methods to detect PCV2 antibodies, since the titre obtained can be interpreted in a biological sense (2). Nevertheless, the method is relatively complex, depending on the availability of cell culture, viable virus and technical expertise. Therefore, the replacement of IPMA for alternative serological tests such as ELISA is desirable.

The objective of this study was to correlate the antibody titres obtained by IPMA with the results obtained from a commercial blocking ELISA.

Materials and methods

A total of 908 pig sera were tested by a previously published IPMA protocol (3). The IPMA technique was carried out making serial four-fold dilutions of sera, from 1/20 to 1/20480. Serological titres were transformed to log_2 value.

Samples were also analysed by a commercial blocking ELISA (Serelisa®PCV2 Ab Mono Blocking, Synbiotics®) using the quantitative method of a single dilution, following manufacturer's instructions. The ELISA results were expressed as: 1) antibody titres (according to the mathematical formula provided by the manufacturer), and 2) the reciprocal optical density (OD) value (recOD) (calculated by subtracting the inverse of the OD negative control to the inverse of the OD of the sample).

The correlation between IPMA and ELISA titres was analyzed in two ways. Firstly, it was calculated the mean ELISA values for each IPMA titre, and the corresponding lineal equation was adjusted. Secondly, the 95% confidence intervals (CI) of each IPMA were calculated. Subsequently, a value of IPMA was possible to be inferred by each ELISA result obtained (recOD or titre).

Results

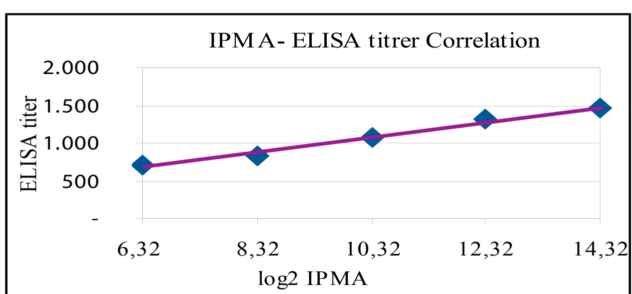
Table 1 summarizes the IPMA results and the corresponding average values of ELISA recOD/titres.
The 95% CI was narrow for each IPMA titre. The only exception was seen in the 4.32 IPMA log_2 titre, which had a wide CI (probably because of the low number of individuals into this group), and therefore it was excluded from the analysis.

Table 1. Log_2 PCV2 IPMA titres and corresponding ELISA recOD/titres with confidence intervals (CI).

n	IPMA titre	recOD	CI	ELISA titre	CI
7	4.32	0.4012	±0.3924	712	±602
56	6.32	0.3584	±0.1158	718	±215
165	8.32	0.3787	±0.0646	818	±116
235	10.32	0.5271	±0.0576	1067	±114
186	12.32	0.6392	±0.0599	1316	±128
259	14.32	0.6929	±0.0491	1452	±106

The lineal correlation (R^2) between IPMA titres (log_2 6.32 to 14.32) and the results of the ELISA test was higher than 0.98 for recOD values and titres (Figure 1).

Figure 1. Correlation between IPMA and ELISA titres



Discussion

Obtained results indicated a linear relation between IPMA titre and ELISA recOD/titrer (R^2>0.98), with the exception of the group of sera with the lowest IPMA titre. Therefore, it seems that the commercial blocking ELISA test provides a predictive value similar to that offered by the IPMA. For this reason, the ELISA test seems to be a reliable potential substitute of IPMA to monitor antibody responses to PCV2 infection and/or vaccination.

Acknowledgements

M. Mora and C. García technical assistance is highly appreciated.

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P.038

USE OF A COMMERCIAL INDIRECT ELISA TEST FOR PORCINE CIRCOVIRUS TYPE 2 (PCV2) TO INFER IMMUNOPEROXIDASE MONOLAYER ASSAY (IPMA) PCV2 SEROLOGICAL TITRES

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Introduction

Serology is frequently used to determine antibody presence/absence or amount of antibodies against a given pathogen. The appropriate use of serological techniques should allow determining infection dynamics and optimal timing of vaccination/medication. Therefore, serology must be considered a very practical tool for field veterinarians.

With the advent of porcine circovirus type 2 (PCV2) vaccines, the interest for antibody monitoring has substantially increased during last 5 years. In fact, a number of serological assays to detect PCV2 antibodies are already available. First developments included those based on cell cultures infected with PCV2, namely immunoperoxidase monolayer assay (IPMA) and immunofluorescent antibody assay (IFA). The great advantage of those methods is the feasibility of titration, so, a biological titre-dependent interpretation can be established (1). However, these techniques are mainly used in research and its application to a high number of samples is expensive, time-consuming and there is a need for cell culture and virological expertise. Therefore, the subsequent development of ELISA techniques solved part of these problems, allowing the possibility of working with high number of samples in an easy-to-apply platform. However, ELISA techniques are not usually used for titration but for a qualitative result.

Based on above mentioned premises, the present work was aimed to interpret PCV2 serological values of a commercial ELISA as a quantitative technique by means of comparison with IPMA.

Materials and methods

A total of 645 pig sera were tested by a previously described IPMA technique (2) as well as by a commercial indirect ELISA test (INGEZIM® CIRCO IgG, Ref: 11.PCV.K.1) in order to detect PCV2 antibodies. The IPMA technique was performed in 4-fold dilution series, so, different potential antibody titres were possible: 1/20, 1/80, 1/320, 1/1280, 1/5120 and 1/20480. Serological titres were then transformed to $\log_2$ titres. The ELISA technique offered an optical density (OD) value, and although an OD cut-off value was given by the manufacturer, this was not considered for the purpose of this study. All sera were tested in duplicate by the ELISA technique.

The correlation between IPMA and ELISA titres was analyzed in two ways. Initially, mean ELISA values for each IPMA titre were calculated, and corresponding linear and polynomial equations were adjusted. In a second step, the 95% confidence intervals (CI) of the ELISA OD values per each IPMA titre were computed. Overall, it was possible to infer the IPMA titre based on results of ELISA OD values.

Results

A summary of PCV2 IPMA titres and the mean ELISA OD values plus 95% CI are given in Table 1. Correlation of both values was high when considered it as linear (R^2=0.88) and very high when considered as polynomial (R^2=0.99). In the latest case, the formula $y = 0.0166x^2 – 0.1738x + 0.7813$ allowed inferring a PCV2 IPMA antibody titre (x) knowing the corresponding ELISA OD value (y).

Table 1. Log_2 PCV2 IPMA titres and their corresponding mean ELISA OD value (plus 95% confidence intervals, CI) of the studied sera.

$\log_2$ IPMA	Mean OD value	95% CI
4.32 (n=58)	0,3366	±0,0599
6.32 (n=160)	0,3650	±0,0330
8.32 (n=123)	0,4948	±0,0526
10.32 (n=73)	0,6719	±0,0743
12.32 (n=75)	1,2698	±0,0830
14.32 (n=156)	1,6981	±0,0760

Discussion

The ultimate goal of this study was to explore the potential usage of an indirect ELISA test able to detect PCV2 antibodies to provide a quantitative value similar to that offered by the IPMA technique. Since this latter technique allows titration and possibility of interpretation in a biological sense (1), the present study supports the notion of using an indirect ELISA technique to predict IPMA titres with high degree of correlation. In consequence, and especially important for field veterinarians using serology for PCV2, this indirect ELISA can be applied for monitoring PCV2 infection and vaccination under field conditions and be interpreted as a quantitative technique in a similar manner than IPMA.

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P.039

EVALUATION OF THREE DIFFERENT ELISA TESTS FOR THE DETECTION OF HIGH LEVELS OF MATERNALLY DERIVED PCV2 ANTIBODIES AS MEASURED BY IMMUNOPEROXIDASE MONOLAYER ASSAY

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Introduction

With the introduction of PCV2 vaccination, there is an increasing need to monitor the PCV2 serological profiles of young pigs in individual herds to assess the optimal time for vaccination. The immunoperoxidase monolayer assay (IPMA) technique has been extensively used for the detection of PCV2 antibodies. However, for practical reasons, the use of IPMA for herd profiling on a large-scale is not feasible. The objective of the present study was to test the ability of three different PCV2 ELISA tests to detect maternally derived antibodies (MDA) against PCV2, and evaluate them as potential alternatives to the IPMA technique for discriminating between high (IPMA > 1280) and low-medium (IPMA ≤ 1280) PCV2 MDA titers (classification after Rodríguez-Arrioja et al. (1)).

Materials and methods

Samples: Serum samples from non-vaccinated 3 to 4 week-old pigs from three different European farms were analyzed by IPMA. The technique was performed at four-fold dilutions (20-20480) and its detection limit was 1:80. For each IPMA titer (≤ 80, 320, 1280, 5210 and 20480) 20 samples were selected (Total n=100).

ELISA tests: All samples were analyzed by means of two quantitative ELISA tests, Ingezim® Circo IgG (Ingenasa, Madrid, Spain) and Serelisa® PCV Ab (Synbiotics, Lyon, France), and by an in-house semi quantitative ELISA developed by the Danish National Veterinary Institute. The two commercial ELISAs were performed as recommended by the manufacturer and third one as described elsewhere (2).

Data analyses: Using IPMA as the gold standard, a receiver operating characteristic (ROC) curve was built to evaluate the ability of each ELISA test to correctly classify the samples as having high (IPMA > 1280) or low-medium (IPMA ≤ 1280) levels of PCV2 MDA. For each decision threshold, the ROC curve provided a combination of sensitivity and specificity for positive classification (IPMA titer >1280). For each pair of ELISA and its potential cut-off, the agreement beyond chance with the IPMA results above or below the cut-off was calculated and expressed as the kappa statistic.

Results and discussion

For each of the three ELISA tests, the area under the ROC curve (AUC) was close to 1, indicating good discrimination between high and low-medium PCV2 MDA levels (Table 1)

Table 1: Area under the ROC curve for each ELISA test

ELISA test	AUC	*p-val*	95% CI
Ingezim® Circo IgG	0.925	<0.01	0.882 - 0978
Serelisa® PCV Ab	0.928	<0.01	0.872 - 0.984
In-house Denmark	0.932	<0.01	0.886 - 0.979

Based on the coordinates of ROC curve (pairs of sensitivity/specificity resulting from varying the ELISA threshold over the whole range of titers), three potential cut-offs were selected for each ELISA test. For each of them, the agreement beyond chance with the IPMA results was calculated. As seen in table 2, the highest agreement with the categorization of MDA levels as done by IPMA was observed with Serelisa (cut-offs 500 and 1000).

Table 2: Potential cut-offs for detecting high levels of PCV2 MDA in each ELISA test, with their corresponding sensitivity, specificity and agreement with IPMA.

ELISA	Potential cut-off[1]	Sensitivity	Specificity	*K*
Ingezim® Circo IgG	500	0.80	0.88	0.68
	1000	0.65	0.93	0.60
	2000	0.48	0.97	0.47
Serelisa® PCV Ab	500	0.85	0.90	0.75
	1000	0.80	0.93	0.74
	2000	0.60	0.97	0.62
In-house Denmark	250	1.00	0.53	0.48
	1250	0.90	0.83	0.71
	6250	0.53	0.66	0.60

[1] Positive (IPMA >1280) if equal or greater than the cut-off.
K: kappa statistic

Conclusions

Ingezim® Circo IgG, Serelisa® PCV Ab, and the In-house Danish ELISA may be good alternatives to IPMA for the detection of high PCV2 MDA antibodies. The three tests exhibited a comparable accuracy and, in all cases, there were several cut-offs giving acceptable sensitivity and specificity.

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P.040

DETECTION AND QUANTIFICATION OF PORCINE CIRCOVIRUS TYPE 2 (PCV2) AND PCV2-ANTIBODIES IN ORAL FLUID FROM FINISHER PIGS

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Introduction

Application of cotton ropes for collection of oral fluid in pigs for detection of PRRSV has recently been reported (1). This procedure may also offer an alternative to serum for obtaining Porcine circovirus type 2 (PCV2) herd profiles. The objective of the current study was to evaluate cotton ropes for collection and detection of PCV2 specific antibodies and DNA in oral fluid at pen level in finisher pigs.

Materials and methods

In a Danish PCV2 positive finishing herd 50 pens were selected by random sampling. The pens contained on average 23 pigs (range: 5 – 33) with an average weight of 60 kg (range: 30 – 80 kg.). In each pen oral fluid samples were collected by hanging a cotton rope for 30 minutes and letting the pigs chew on it. Faecal pen samples were obtained from each pen by collecting pooled faeces in the corners of the pens at the slatted floor. In each pen blood samples were obtained from 5 pigs selected by systematic random sampling.

Oral fluid was extracted from the ropes by wringing it and all samples were stored on ice immediately after collection. Serum was separated from the blood samples by centrifugation and pooled at pen level before examination. Oral fluid and serum samples were examined for PCV2 specific antibodies by blocking ELISA (ELISA). Oral fluid, serum and faecal pen samples were examined for PCV2 DNA by quantitative real time PCR (qPCR).

Assay validation for ELISA and qPCR on oral fluid was performed prior to examination of collected samples.

Results of oral fluid, pooled serum and faecal pen samples were compared. Pen level diagnostic sensitivity (SE), specificity (SP), positive predictive values (PPV) and negative predictive values (NPV) was calculated for oral fluid and faecal pen samples using the result of the pooled serum samples for classification of each pen. Serial SE, SP, PPV and NPV were calculated for the combination of oral fluid and faecal pen samples (both tests needed to be positive for a pen to be classified as positive).

Results

Both assay validations demonstrated the need for optimization for oral fluid testing, although only slight modifications were sufficient for oral fluid to be processed as serum. All pens were serum ELISA positive while only 54% were ELISA positive in oral fluid samples. Antibodies were not detected in any oral fluid samples from pens with pooled serum titers of 125 or less (n=19). Further, 38% of pens (n=8) with pooled serum titer = 625 and 1 pen (n=19) with pooled serum titer = 3125 were negative in the oral fluid samples.

A total of 80% of the pens were classified as PCV2 positive by qPCR in pooled serum samples. For both oral fluid and faecal pen samples SE = 0.98, SP = 0.60, PPV = 0.91 and NPV = 0.86. Serial testing provided SE = 0.95, SP = 0.84, PPV = 0.96 and NPV = 0.81. The mean difference between qPCR results in oral fluid and pooled serum samples was 2.0 log10 copies (standard deviation (sd) = 1.34). For faecal pen and pooled serum samples the difference was 1.60 log10 copies (sd = 1.33).

Discussion

Results of the current study demonstrate that PCV2 antibodies and DNA can be detected in oral fluid collected by cotton ropes. High titers of serum antibodies were necessary to obtain positive oral fluid samples. This may limit the application of the procedure for serological herd profiling. The low SP for oral fluid may reflect false positive samples or a higher sensitivity at pen level compared to obtaining 5 serum samples. Applying serial testing of oral fluid and faecal pen samples provided results comparable to obtaining 5 serum samples in a pen.

The quantitative results of the qPCR in the oral fluid samples demonstrated a high level of consistency compared to pooled serum samples. The level in oral fluid was 2.0 log10 copies higher than in pooled serum samples. Interestingly faecal pen samples demonstrated almost identical results to oral fluid. This may imply faecal contamination of the cotton ropes.

In conclusion, collection of either oral fluid by ropes or faecal pen samples offers a reliable procedure for detection and quantification of PCV2 in finishing herds.

Acknowledgements

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P.041

USE OF THE NEW BACUCHECK™ ELISA TO DIFFERENTIATE BETWEEN PCV2-VACCINATED AND UNVACCINATED PIGS OF DIFFERENT AGES

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Introduction

Porcine circovirus type 2 (PCV2) has emerged as a severe problem for the swine industry all over the world. Although various vaccines have proved highly effective (1), antibodies against PCV2 can be detected in most pigs in the field. Individual immunity appears to be important for herd protection, but it has been difficult to differentiate between antibodies to vaccinal and field antigens. Furthermore, background maternal derived antibodies and field exposure may complicate the interpretation of PCV2 serology.

A new ELISA kit (BacuCheck™ ELISA) has recently been developed to provide proof of vaccination with Porcilis® PCV (Intervet/Schering-Plough). The kit detects antibodies against bacoluviruses, which are used in the production of the structural capsid protein ORF2 component of vaccines. The aim of the present study was to measure antibody levels over a period in Porcilis® PCV-vaccinated and unvaccinated pigs using the BacuCheck™ ELISA.

Materials and methods

In total, 40 piglets from a 1,000 sow farrow-to-finish farm were allocated between two groups. One group was vaccinated with a single dose of 2 ml Porcilis® PCV at 21 days of age, while the other was left unvaccinated to act as controls. Blood samples were collected at 7, 11, 14 and 18 weeks of age. All sera were tested with the BacuCheck™ ELISA as well as with the Synbiotics Serelisa®, a Mono Blocking ELISA to detect and quantify PCV2 antibodies.

Results

The percentage of positive sera and the mean S/P ratio by BacuCheck™ ELISA for both groups are shown in Figure 1. In the vaccinated group, more than 90% of the samples were positive at all ages. The mean S/P ratio for this group remained at around 0.6 (0.51–0.68). The sera of all unvaccinated pigs at 7 weeks of age were negative, but the percentage of samples positive to the BacuCheck™ ELISA increased over time. Figure 2 shows the mean antibody titres obtained from the Synbiotics Serelisa®. At 7 weeks old, vaccinated pigs had a mean titre of 3,441, compared with 1,118 in the unvaccinated pigs. Specific PCV2 antibody titres in the group of vaccinated pigs were still increasing after 7 weeks of age, but in the controls they did not rise until week 14.

Figure 1: Percentage of vaccinated and non vaccinated pigs positive by the BacuCheck ELISA and mean S/P ratio per group.

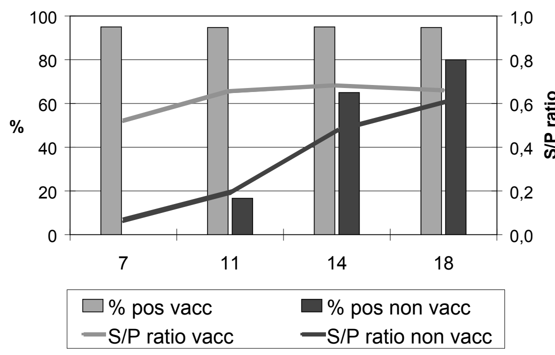
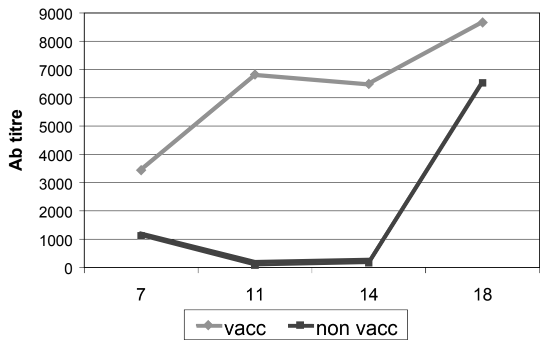


Figure 2: Mean antibody titre obtained by the Synbiotics Serelisa® for each group.



Discussion

Combining the results from the BacuCheck™ ELISA and the Synbiotics Serelisa® clearly differentiated between Porcilis® PCV-vaccinated and unvaccinated groups at 7 weeks of age. A mean S/P ratio <0.3 with less than 80% of pigs positive to the BacuCheck™ ELISA, together with mean specific PCV2 antibody titres <1800 in the Synbiotic Serelisa® clearly confirms, at group level, that the piglets had not been correctly vaccinated with 2ml Porcilis® PCV.

Moreover, the results indicate that pigs do contract baculoviruses during the fattening period, which is in line with the authors' experience obtained from the analyses of field samples. The BacuCheck™ ELISA should therefore only be recommended for use in pigs up to 12 weeks of age.

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P.042

SERUM PROTEIN PROFILES BASED ON SELDI-TOF MS AS BIOMARKER FOR INFECTIOUS DISEASE STATUS IN PIGS

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Introduction

In veterinary medicine, there is a need for early warning tools for disease (1,2). Infections with Porcine Reproductive and Respiratory Syndrome virus (PRRSV) and Porcine Circovirus type 2 (PCV2) belong to the most relevant endemic pig infections and early disease diagnosis can support veterinary decision. In this study the potential of serum protein profiles based on SELDI-TOF MS as biomarker for infectious disease status is explored in an experimental infection model (3).

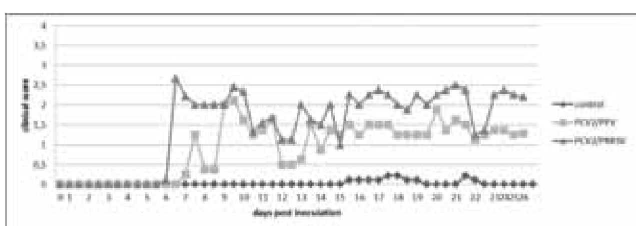
Materials and methods

Serum samples were analyzed from 26 pigs in an experimental model for Porcine Circovirus type 2 (PCV2) infection in combination with either Porcine Reproductive and Respiratory Syndrome virus (PRRSV) (n=9) and Porcine Parvovirus (PPV) (n=8) and from non-infected control animals (n=9).
SELDI-TOF MS protein profiles were generated from serum samples taken at different time points, before infection (day 0), before clinical signs became apparent (day 5) and at day 19, when animals showed clear clinical signs as a result of the infections. For statistical analysis, SELDI-TOF proteomics data were grouped on basis of levels of significance. The analysis of a total of 586 proteins was performed using ridge partial least square regression and ANOVA.

Results

All pigs in the PCV2/PRRSV infected group developed clinical signs of disease, which started to appear form day 6 or 7 onwards and lasted throughout the study. In the PCV2/PPV group the start of symptoms was later, but also in this group all pigs developed disease. Major signs of disease were respiratory distress, which was more severe in the PRRSV infected group. The typical enlargement of inguinal lymph nodes was observed in all PCV2/PRRSV infected pigs and in 20% of the PCV2/PPV infected pigs.

Figure1: Mean clinical score on days post infection



Based on protein profiles, classification accuracy of infected versus non-infected animals was very good. At day 5 and 19 post infection, 88% and 93% respectively of infected animals were identified as such. Moreover, protein profiles could distinguish between separate combinations of pathogens, although results for PCV2/PRRSV infected animals were slightly better compared to PCV2/PPV infected animals (Table 1). Limiting the number of proteins in the profile generally had minor effects on the classification performance.

Table 1. Contingency table with the number of animals for each class of actual infection status, i.e. based on the experimental infection and predicted infection status, i.e. based on protein profile at day 5 and day 19, when using 50 preselected proteins on combined data.

		Day 5[1]			Day 19[1]		
		Actual status			Actual status		
		PCV2/PPV	Control	PCV2/PRRSV	PCV2/PPV	Control	PCV2/PRRSV
Predicted status	PCV2/PPV	2	2	1	5	3	4
	Control	4	7	0	1	4	0
	PCV2/PRRSV	1	0	8	2	0	3

1. The p-value of the one-sided Fisher exact test is <0.001 and 0.095 at day 5 and 19 respectively.

Discussion

Results from this survey, using data from standardized experimental settings, show that protein profiles based on Seldi-tof MS technology can detect viral infection in pigs in early phase of the disease. Application of Seldi-tof proteomics data may have potential for development of biomarkers for disease, but need further exploration in field settings.

Acknowledgements

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P.043

HAPTOGLOBIN SERUM CONCENTRATION IS NEGATIVELY CORRELATED WITH LYMPHOID DEPLETION IN PMWS AFFECTED PIGS

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Introduction

Acute phase proteins (APP) are a group of plasmatic proteins which concentration change after infection, inflammation or surgical trauma, factors that trigger the acute phase response (APR) (1). Serum concentration of Haptoglobin (Hp) increases during the APR and it is a useful marker of clinical disease in pigs (2).

Porcine circovirus type 2 (PCV2) is ubiquitous in swine populations. The PCV2 associated disease known as Postweaning Multisystemic Wasting Syndrome (PMWS) is considered as a major economic problem for the global swine industry. Immunohistologic and pathologic studies suggest that pigs with PMWS may be immunosuppressed. Lymphocyte depletion of follicular and interfollicular areas and inflammatory infiltration by histiocytes of lymphoid tissues is a constant lesion of PMWS affected pigs (3).

The aim of this study was to assess the relation between the Hp concentration in serum and the degree of lymphoid depletion in wasted pigs clinically diagnosed as PMWS.

Materials and methods

Mediastinic, traqueobronquial, inguinal and mesenteric lymph nodes, tonsil and spleen from 50 wasted pigs were fixed in formalin, embedded in paraffin and stained with haematoxylin and eosin for histopathological examination. The criteria considered for the diagnosis of PMWS were those previously described (4). The Lymph node depletion was categorized in grades from 0 to 3. Grade 0 was used for no depletion, grade 1 for low depletion in more than two different lymphoid tissues, grade 2 for moderate lymphoid depletion in more than two lymphoid tissues and grade 3 for severe lymphoid depletion in more than two different tissues. Whole blood was obtained and serum levels of Hp were quantified by commercial ELISA kit (Tridelta, Ireland). Pearson´s correlation and Test of Mann-Whitney's U were performed.

Results

Hp concentrations depending on lymphocyte depletion score are represented in table 1. Hp levels presented statistical differences between pigs with no depletion and pigs with severe depletion (p = 0.001), and between pigs with low and severe depletion (p = 0.014).

Table 1: Concentration of Hp ± standard deviation for each lymphocyte depletion score.

Lymphoid depletion	Hp (mg/ml)
None (n = 16)	178.6 ± 40.3
Low (n =12)	138.2 ± 50.1
Moderate (n = 9)	41.4 ± 36.4
Severe (n = 13)	4.4 ± 0.3

There was an inverse correlation between Hp blood concentration and lymphoid depletion severity (r = -0.47, p = 0.001).

Discussion

Hp captures hemoglobin in order to prevent both iron loss and hemoglobin-mediated renal parenchymal injury during hemolysis (5). Decreased serum Hp concentrations are reported in cases of hemolysis and iron deficiency (6). PMWS affected pigs have pallor and microcytic hypochromic anemia with a decrease of serum iron (7) which could explain why Hp serum concentration in pigs with severe lymphoid depletion caused by PMWS was lower than in pigs with none or low depletion.

The APR is triggered by IL1, IL6 and TNFα (1). It has been demonstrated that T CD4+ lymphocytes are able to produce IL6 and TNFα (8). In PMWS affected pigs T cells are diminished in follicular areas, mainly affecting the CD4+ cells (7). Human Immunodeficency Virus (HIV) leads also to CD4+ T cell depletion. It has been reported that that many HIV infected human show a reduction in the serum Hp (9). One hypothesis could be that the higher the severity of lymphoid depletion, the lower the levels of IL6 and TNFα, producing as a final result the reduction of Hp synthesis. Obtained data suggest that lymphoid cells number influence serum concentrations of Hp.

Acknowledgements

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P.044

A MULTIPLE SYBR GREEN I REAL-TIME PCR SYSTEM FOR THE SIMULTANEOUS DETECTION OF VIRAL AGENTS AFFECTING INTENSIVE SWINE PRODUCTION

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Introduction

Under current conditions of intensive swine's production, it is possible for pigs to be simultaneously infected with two or more viral pathogens (1). Thence, the aetiology diagnosis from clinical signs is nowadays considered a very difficult task. The real-time PCR based on SYBR Green I melting curve analysis has been one of the most effective techniques employed for the detection and identification of various viral pathogens (2). Hence, the current study was aimed to the development, optimization and evaluation of a multiple real-time PCR SYBR Green I system couple to a melting curve analysis for the detection and differentiation of PCV-2, PRV, PPV TTSuV1 and TTSuV2 in a single run.

Material and methods

The multiple real-time PCR assays was accomplished: (i) an initial incubation by 10 min at 95 ◦C was followed by (ii) 40 cycles of denaturation at 95ºC for 10 sec., a touchdown annealing from 60ºC to 55ºC for 5 sec. with a step size of 0.5ºC and step delay of 1 cycle, and elongation at 72ºC for 20 sec. with acquisition of fluorescent data. After the PCR cycles, a melting curve was generated. Virus used in this study included, a Cuban PCV-2 isolate PNE1 (GenBank accession no. FM999737) (3), PRV reference strain NIA-3, a Cuban PRV isolate V208 (4) PPV reference strain NADL-2 and a Cuban PPV isolate E6 (5). The effect of different tissue sample types (matrix effect) on the analytical sensitivity, on cycle threshold values (Ct-value) and Tm-values was assessed. The impact of the melt rate on the resolution of peak of the melting curves was assessed. Thus, three different melt rates 0.1ºC/sec., 0.4ºC/sec. and 0.8ºC/sec. were tested. Finally, to assess the performance of the multiple real-time PCR assay on clinical specimen a total of 40 tissues (spleen and lymph nodes) samples collected in a parallel study were used (6).

Results

The multiple real-time PCR system was integrated by two duplex real-time PCRs (i-PCV-2/PRV and ii-TTSuV1/PPV) as well a single real-time PCR reaction for TTSuV2, all performed in a single-run. The detection limit of the multiple real-time PCR system was 18 DNA copies for TTSuV1, 20 for TTSuV2 and 25 for PPV, PRV and PCV-2 respectively. The specific Tm-values of the multiple real-time PCR system assay were decreased when serum and tissue samples (lung and spleen) were used as matrixes $p<0.05$. Likewise, the Ct-value for multiple real-time system were higher when serum and tissue samples (lung and spleen) were used. Different resolutions on Tm-peaks were obtained when different melt ramp times (ºC/sec.) were assessed on acquisition of fluorescent data in the melting curve analysis. The best resolutions on Tm-peaks were obtained when a melt ramp time of 0.4ºC/sec was evaluated. The multiple real-time PCR system was able to detect and well-discriminate even though 2 or more viral agents were present in the tissue samples and it showed a 100% of preliminary diagnostic sensitivity and specificity.

Discussion

The aetiology of the respiratory and reproductive syndromes as well as of the multifactorial diseases that affect the pigs is a complex task for the veterinary services. Current evidences have shown that co-infection of pigs with PCV-2 and other viral pathogens such as PPV and PRV resulting in more severe clinical signs of PCVD. Likewise, recent studies suggests that Torque teno sus virus species 1 (TTSuV1) and 2 (TTSuV2) have been linked to pathological conditions related with PMWS (7) and the porcine respiratory disease complex (8). Hence, the laboratory diagnosis is indispensable for clarify which agents are causing these illness. Concerning the laboratory tests the real-time PCR method has provided several advantages over other confirmatory methods such as: viral isolation, ELISA or conventional RT-PCR (9). Here, a validated real-time PCR based on SYBR Green I detection coupled to melting curves analysis for the detection and differentiation of PCV-2/ PRV/TTSuV1/PPV and TTSuV2 in the same clinical sample is proposed.

The analytical and diagnostic performance as well as the repeatability of the multiple real-time PCR system obtained from the assessment on field samples allows the use of this assay for the diagnosis of laboratory or could be a useful tool in epidemiological studies of respiratory, reproductive or another multifactorial diseases syndrome in which PCV2, PPV, PRV, TTSuV1 or TTSuV2 might be involved.

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P.045

POWERS AND PITFALLS OF PORCINE CIRCOVIRUS TYPE 2 QUANTIFICATION BY REAL-TIME QUANTITATIVE PCR

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Introduction

Porcine circovirus type 2 (PCV-2) is one of the most abundant viral pathogens of swine and still has tremendous financial impact on the swine industry. As with other infectious diseases, the severity of clinical symptoms associated with PCV-2 infection is related to the amount of virus present in the tissues of the affected animal. Determination of PCV-2 load by real-time quantitative PCR (qPCR) may therefore be an attractive and valuable tool for differentiating porcine circovirus associated disease (PCVAD) from abundant subclinical infection. A PCV-2 viral load exceeding 1.00E+07 copies per 500 µg of tissue, or 1 ml of serum, respectively, has been shown to correlate with clinically manifested PCVAD (1-2). Clearly, viral load determination depends on the accuracy of the methods used for this purpose. Over the recent years, a plethora of PCV-2 qPCR assays has been published, using different chemistries, and targeting different regions of the PCV-2 genome (1-5). Moreover, different DNA standards were used for quantification of PCV-2 load, making direct comparison of results difficult. This demonstrates an urgent need for cross-validation and proficiency testing of the methods currently in use.

By comparing several different qPCR methods and chemistries, we show that qPCR method selection may significantly influence the outcome of PCV-2 quantification.

Materials and methods

The complete PCV-2 coding sequence was inserted into a commercial plasmid vector and served as single DNA standard for PCV-2 DNA quantification, using three previously published qPCR methods (2-4) and a novel SYBR-Green assay. All four assays target different regions of the PCV-2 genome within either ORF1 or ORF2. PCV-2 loads in pig tissues, serum and boar semen were estimated and compared between the different methods tested.

Results

A single plasmid DNA standard was employed to allow optimal comparability of the different PCV-2 qPCR methods. All tested qPCR assays were able to detect the PCV-2 full genome DNA standard with high sensitivity and complied with up to date quality requirements for qPCR regarding standard curve correlation and qPCR efficiency (6). Quantification of PCV-2 DNA loads in porcine clinical tissues, serum and boar semen using four different qPCR methods resulted in highly different estimates of absolute PCV-2 loads between individual assays. In fact, the difference between the lowest and the highest PCV-2 load estimates of the same sample exceeded a factor of thousand in some cases.

Discussion

A recent North America-centred PCV-2 ring test has shown major differences in the absolute sensitivity of PCV-2 qPCR assays used by participating laboratories (7). In another inter-laboratory comparison, systematic differences in viral load estimation between two methods have been described (8). The reasons for these variations were unknown, or assigned to differences in qPCR chemistries or choice of PCV-2 genome target region (8). Collectively, these studies illustrate the importance of cross-validation of the different PCV-2 qPCR assays currently used for this purpose.

We show here that *state of the art* PCV-2 qPCR assays with virtually identical analytical sensitivities and qPCR kinetics result in highly divergent estimates of PCV-2 burden, even when the same DNA standard is used for quantification. Our data indicate that the target region within the PCV-2 genome is unlikely to be of major significance to the outcome of viral load estimation. In contrast, we suggest that increasing PCV-2 genome diversification is responsible for the discrepancies in the viral load estimates observed in our experiments. Our results demonstrate the necessity of careful and continuing PCV-2 qPCR validation for both qualitative and quantitative detection of PCV-2 in clinical samples. Furthermore, the developed PCV-2 full genome standard offers the possibility to cross-validate existing PCV-2 qPCR assays and to organize PCV-2 qPCR proficiency testing.

Acknowledgements

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P.046

A COMPARISON OF IMMUNOHISTOCHEMISTRY AND IN SITU HYBRIDIZATION FOR THE DETECTION OF PORCINE CIRCOVIRUS TYPE 2 IN PIGS

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Introduction

Porcine circovirus type 2 (PCV2) is an etiological agent of postweaning multisystemic wasting syndrome (PMWS) (1). To confirm PMWS the specific criteria must be fulfilled (4). The aim of the study was to develop and to optimize immunohistochemistry (IHC) method for PCV2 identification and to compare it with *in situ* hybridization (ISH) method.

Material and methods

The study was conducted on formalin-fixed, paraffin embedded samples of internal organs collected from 2008 to 2010 from wasted swine suspected of PMWS. Overall, 44 sections (38 lymph nodes, 5 intestines and 1 thymus) previously analyzed by ISH, were tested by IHC for the presence of PCV2.

Avidin-biotin complex immunoperoxidase method was developed for PCV2 identification. ISH was performed on corresponding slides as described before (3; 5). All the samples were also hematoxylin-eosin (HE) stained.

Results

PCV2 was detected in most tissues subjected to the analysis. After IHC positive staining was observed in cytoplasm of macrophages and multinucleated giant cells in lymph nodes. In small intestine the staining was observed in gut-associated lymphoid tissue and in villous epithelium.

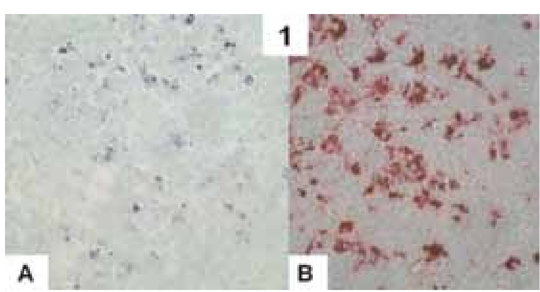
All 4 sections identified by ISH as highly positive gave similar results by IHC. Also, all experimental samples negative in ISH showed no staining in IHC. However, there were differences in scoring of the sections after staining with the two compared methods. In 21 slides (47,7%) stronger staining was found in IHC than in ISH. Of these, 6 slides that scored doubtful in ISH, were found clearly positive in IHC (Fig. 1).

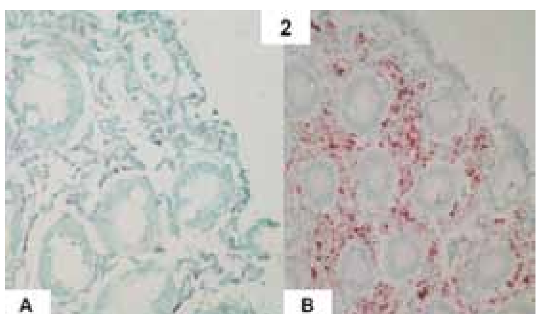
Discussion

According to Sorden's criteria, IHC and ISH are the only laboratory methods fulfilling the requirements of PMWS diagnosis (4).

In this study IHC protocol was developed for detection of PCV2 antigen in tissues from pigs with PMWS. The results demonstrate that both ISH and IHC successfully detected PCV2 viral antigens or nucleic acid in examined tissues. However, our results indicate that IHC has higher sensitivity and specificity than ISH. This is in agreement with other with observations by other authors (2,3).In addition, IHC results are easier to interpret due to better image quality after staining. Summarizing, IHC is reliable and useful technique for PMWS diagnosis and is likely to substitute ISH in our diagnostic laboratory.

Figure 1. Detection of PCV2 by *in situ* hybridization (ISH) (1A, 2A) and immunohistochemistry (IHC) (1B, 2B), in corresponding sections of lymph nodes (A) and intestines (B). PCV2-positive staining is present in ISH and IHC slides in a germinal centre, in cortex-like tissue of a lymph node (A and B) as well as in an intestinal lamina propria (C and D). More intensive staining is visible in immunohistochemistry. X 200





Acknowledgements

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P.047

AN INVESTIGATION OF LUNG LESIONS AND PATHOGENS ASSOCIATED WITH PORCINE RESPIRATORY DISEASE COMPLEX IN KOREA

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Introduction

Porcine respiratory disease complex (PRDC) has an important impact on worldwide swine industry. The most common viral agents involved in PRDC include porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV) and porcine circovirus type 2 (PCV2). It is important to remember that the interaction between pathogens can be an important factor in determining the severity of respiratory disease. Those pathogens that alter or modulate the respiratory immune system will have a greater overall impact when multiple pathogens are present (1).

The purpose of this study was to investigate association with gross lesions and viral agents including PRRSV, SIV and PCV2 in slaughter pig.

Materials and methods,

A total of 500 lung samples were collected randomly from slaughtered pigs in Korea during August through December of 2010. The gross lesions were classified according to the five stages (0-10, 11-20, 21-30, 31-40, ≥41, unit =%) and 20 samples were selected to detect viral pathogens from each stage.

The lung tissues were collected in the part of right cardiac lobe, tissue samples were homogenated and extracted to DNA for PCV2 and to RNA for PRRSV and SIV (QIAamp® DNA Mini Kit and RNeasy® Mini Kit, QIAGEN). PCV2 and SIV were analyzed by real-time PCR, PRRSV were analyzed by nested RT-PCR (Table 1).

Table 1. Primer sequences to detect viuses

PCV2		Forward	CCAGGAGGGCGTTGTGACT
		Reverse	CGCTACCGTTGGAGAAGGA
			FAM-AATGGCATCTTCAACACCCGCCTCT-TAMRA
PRRSV	Outer	Forward	ATGGCCAGCCAGTCAATCA
		Reverse	TCGCCCTAATTGAATAGGTG
	Inner	Forward	CCAGATCGTGGGTAAGATCATC
		Reverse	CAGTGTAACTTATCCTCCTGA
SIV-H1N1		Forward	GCACGGTCAGCACTTATYCTRAG
		Reverse	GTGRGCTGGGTTTTCATTTGGTC
			FAM-CYACTGCAAGCCCA"T"ACACACAAGCAGGCA-BHQ1

Results

The gross lesions which were classifed according to five stage were shown in Table 2.

Table 2. Gross lesions of the lungs in slaughter pigs

Gross lesions (%)	Lung samples	
	n*	%
0-10	156	31.2
11-20	148	29.6
21-30	80	16
31-40	50	10
≥41	66	13.2
Total	500	100

*Number of the lung.

Relationship with gross lesions and virus detections was shown in Table 3. Forty-one samples were not detected PCV2, PRRSV and SIV-H1N1 by PCR, whereas 11 samples were co-infected.

Table 3. Relation with gross lesions and PCR results

Gross lesions(%)		0-10		11-20		21-30		31-40		≥41		
Selected samples		20		20		20		20		20		Total
		n*	%	n	%	n	%	n	%	n	%	n
No viral agent detected		11	55	8	40	9	45	5	25	8	40	41
Infected single viral agent	PCV2	8	40	8	40	7	35	12	60	10	50	45
	PRRSV	1	5	3	15	2	10	3	15	2	10	11
	SIV-H1N1	0	0	3	15	3	15	5	25	3	15	14
	Total	9		14		12		20		15		70
Co-infected	PCV2+ PRRSV	0	0	0	0	0	0	1	5	0	0	1
	PCV2+ SIV-H1N1	0	0	2	10	1	5	4	20	3	15	10
	PRRSV+ SIV-H1N1	0	0	0	0	0	0	0	0	0	0	0
	PCV2+ PRRSV+ SIV-H1N1	0	0	0	0	0	0	0	0	0	0	0
	Total	0		2		1		5		3		11

*Number of detected viral agent.

Discussion

In this study, PCV2 was the most detected virus, and the detection rate of PCV2 was the highest in the range of 31-40%. PRRSV was the fewest detected virus, and the number of detected viral agent was similar to the result from any ranges of gross lesions. The detection rate of viral agents was the highest in the range of 31-40% of gross lesion. The more severe gross lesions increased, the higher the detection rate showed. These results indicate that the PRDC associated with viral pathogens is widely distributed in Korea.

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P.048

PATHOLOGICAL LESIONS OF PORCINE RESPIRATORY DISEASE COMPLEX IN SLAUGHTER PIGS

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Introduction

Porcine respiratory disease complex (PRDC) is caused by the multifactorial pathogens isolated from pigs (1). Within vary pathogens, the most common viral pathogens associated with the PRDC are porcine reproductive and respiratory syndrome (PRRS), swine influenza (SIV) and porcine circovirus type 2 (PCV2).

The purpose of this study was to assess the lung lesions and to compare gross pathology with histopathology in the lungs of slaughter pigs from PRRS, SIV and PCV2 detected farms.

Materials and methods

During August through December of 2010, the 500 lung samples were collected randomly on slaughter pigs from PRRSV, SIV and PCV2 detected farms in Korea.

Percents of the lung lesions in each lobe were calculated by Straw's method (2). Total percent of gross lesions were classified according to the five stages and scored on a scale of 0 to 5 (0%; 0, 1~10%; 1, 11~20%; 2, 21~30%; 3, 31~40%; 4, ≥41%; 5).

Stage of gross lesions of the lung was classified with normal, acute, subacute and chronic lesions (Table 1) (3).

Table 1. Stages of macroscopic lung lesions

Acute	edematous, hyperemic and swollen
Subacute	those in which swelling and hyperemia were no longer present, but in these cases there may still have been mild edema and often marked exudation into the bronchi
Chronic	characterized by atelectic, fibrotic and grey-red lung tissue with dilation of exudate-filled bronchi

For a comparison between gross lesions and histopathology, the 25 lung tissues were collected randomly from each stage on a right cardiac lobe and stained by H&E. BALT (bronchioles and bronchi associated lymphoid tissue) hyperplasia was graded as follows (Table 2) (3).

Table 2. BALT hyperplasia grade by histopathological findings of the lung

0	absent
+	mild diffuse infiltration of lymphocytes in the peribronchial, peribronchiolar and perivascular tissues including the lamina propria of the airways
++	moderate increased diffuse infiltration of lymphocytes and/or presence of a few lymphoid nodules
+++	marked number of lymphoid nodules
++++	extensive number of lymphoid nodules

Data were analyzed statistically by Chi-square test or by Fisher's exact test. All analyses were done with SAS version 9.1 (SAS Institute, Inc., Cary, NC) with significance of P< 0.05.

Results

Total percent of gross lesions and scores were shown in Table 3. Severity of macroscopic lung lesions according to stage was shown in Table 4. Relationship with gross lesion score, stage of lesions and BALT hyperplasia in histopathology were presented in followings (Table 5 and Figure 1).

Table 3. Total percent of gross lesions

Gross lesions (%)	Score	The lung samples	
		n*	%
0	0	59	11.8
1~10	1	107	21.4
11~20	2	141	28.2
21~30	3	79	15.8
31~40	4	48	9.6
≥41	5	66	13.2

* Number of the lung.

Table 4. Severity of macroscopic lung lesions according to stage

Gross lesions	The lung samples	
	n*	%
Normal	24	4.8
Acute	145	29
Subacute	82	16.4
Chronic	249	49.8

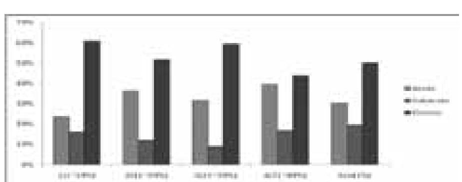
* Number of the lung.

Table 5. BALT hyperplasia associated with stages of macroscopic lung lesions

	Normal (n=25)		**Acute** (n=25)		**Subacute** (n=25)		**Chronic** (n=25)		
	n	%	n	%	n	%	n	%	*p*-value
Absent	13	40.0	5	20.0	2	8.0	1	4.0	0.0001
Mild	12	36.0	15	60.0	4	16.0	3	12.0	0.0003
Moderate	0	0	4	16.0	10	40.0	10	40.0	0.0013
Marked	0	0	1	4.0	7	28.0	7	28.0	0.0038
Extensive	0	0	0	0	2	8.0	4	16.0	0.0503

* Number of the lung.

Figure 1. Relation with gross lesion score and stages of macroscopic lung lesions



Discussion

The most lung lesion score was score 2 in our investigation. Lungs used in this study were a frequent in chronic stage, and it was shown that BALT hyperplasia was more extensive in chronic lesion. These results indicate that PRDC of slaughtered pigs in Korea is chronic type.

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P.049

ATYPICAL GROSS LESIONS AND NECROTIC LYMPHADENITIS ASSOCIATED WITH SEVERE ACUTE PCVAD IN GROWER FINISHER PIGS

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Introduction

Porcine Circovirus type 2 (PCV2) is associated with a spectrum of disease syndromes in pigs, collectively termed Porcine Circovirus-Associated Disease (PCVAD), which have resulted in huge economic losses to the global pig industry in the past decade (3). Diagnosis requires evidence of lymphoid depletion, histiocyte infiltration, loss of lymph node follicular architecture and positive immunostaining. Necrotising lesions in lymph nodes and other tissues have been recorded but are rare (4). This study reports atypical gross lesions associated with necrotising lymphadenitis, splenitis and hepatitis associated with extensive thrombosis induced by PCV2 in grower pigs.

Materials and methods

Acute severe clinical disease featuring rapid wasting, malaise and dyspnoea with high mortality was seen among finisher pigs 2 weeks after entry into a large continuous flow finisher house on a 300 sow farrow to finish unit in the UK. Pigs were immunised at weaning with a chimeric PCV2 and MLV PRRSv vaccines which had replaced a regime of PCV2 sow vaccination 9 months earlier. Four pigs were necropsied to investigate this syndrome.

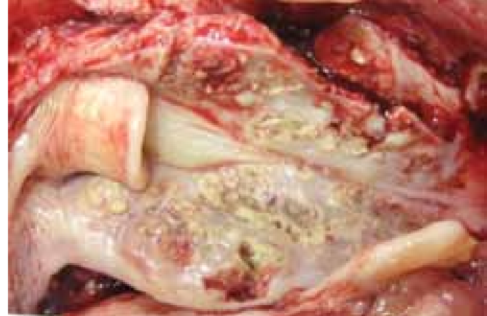
Results

Gross post mortem lesions included necrotic rhinitis (2/4 pigs), necrotic tonsilitis (2/4) [fig. 1], splenic infarcts (3/4), multifocal beige lesions throughout hepatic parenchyma (1/4), and generalised lymphadenopathy (4/4). Classical Swine Fever (CSF) and African Swine Fever (ASF) were ruled out by negative PCR. Aujeszky's Disease (AD) was excluded by negative serology, PCR and virus isolation. A cytopathic effect was seen in PK-15 cell cultures of tonsil and lung and a PCV2b virus, whose nucleotide sequence was unique (in Genbank), was isolated.

Relative to the nucleotide sequence of the isolate with which it shared highest homology there was a single nucleotide change at position 9 of the coding sequence of ORF1 (replicase protein). However, this did not translate to any amino acid differences and the predicted amino acid sequence of the major ORFs (ORF1 replicase; ORF2 capsid, ORF3) were not unique.

Histopathological findings comprised subacute granulomatous lymphadenitis, hepatitis and nephritis with superimposed severe acute multifocal coagulative necrosis, particularly involving the lymph nodes and spleens, associated with thrombosis and haemorrhage. PCV2 immunohistochemistry consistently revealed extensive antigen labelling within lymphoid tissues and necrotic visceral lesions. Although the mucosal necrosis in the tonsil was clearly bacterial in origin, extensive PCV2-specific labelling was present in underlying lymphoid areas.

Figure 1. Necrotic tonsillitis



Discussion

Severe acute PCVAD with vascular lesions resulting in extensive ischaemic necrosis is a rare but recently reported feature of PCVAD (Segales *et al.* 2004). It is still unclear what triggers this clinically acute manifestation; PCV2-induced apoptosis (Kim & Chae 2005) and hypertrophy of high endothelial venules have been suggested as mechanisms (Galindo 2010). Differences in viral isolates are unlikely to explain it. Gross lesions may resemble ASF, CSF and ADV which were all differential diagnoses ruled out in this case. Prophylactic vaccination with a chimeric vaccine did not prevent diseases in this herd. Despite major advances, gaps in understanding of PCVAD pathogenesis remain.

Acknowledgements

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P.050
THE AETIOLOGICAL ROLE OF PORCINE CIRCOVIRUS TYPE 2 IN ACUTE DIARRHOEA OF WEANERS IN DANISH HERDS

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Introduction

Porcine circovirus type 2 (PCV2) has been considered aetiologically involved in the porcine enteritis complex (1).

The objective of the current study was to investigate the occurrence of PCV2 in pigs with and without diarrhoea during outbreaks of acute treatment indicated diarrhoea in pigs between 10 and 70 days post weaning.

Materials and methods

A case control study was conducted. Herds were selected by multistage sampling. All herds serviced by six specialized swine veterinarians from the same vet practice at Zealand and fulfilling the inclusion criteria were selected. The criteria were recurring therapeutic use of in-feed or in-water medication for diarrhoea at room level in pigs between 10 and 70 days post weaning. Only herds representing modern intensive production systems were selected. One outbreak of acute diarrhoea was investigated in each herd. All herds were visited the day following notification from the farmer/veterinarian of an acute treatment requiring outbreak of diarrhoea and the farmer was not allowed to medicate before the pigs were examined. If the pigs had received antibiotic batch medication within the last 7 days of the examination day, the outbreak was excluded from the study.

A sample of 80 pigs in each herd was selected by systematic random sampling among all pigs in the nursery room where the outbreak of acute diarrhoea occurred. The selected pigs were subjected to a clinical examination and faecal samples were collected. Among the examined pigs a simple random sample of 8 pigs with diarrhoea and 8 pigs without diarrhoea was selected and euthanized. Immediately after euthanasia tissue samples were obtained from jejunum, ileum and colon.

Necropsy was performed at DTU-VET the following day. Tissue samples were obtained from apparent gross lesions and the mesenteric lymph nodes. All tissues samples were examined for PCV2 by immunohistochemistry (IHC) and scored on a scale from 1 to 4. Faecal samples were examined for PCV2 by quantitative PCR (qPCR). Dry matter (DM%) was determined for the faecal samples. DM% $\leq$ 18 was considered as diarrhoea (1) and was used for final classification of pigs as diarrheic (cases) or non-diarrheic (controls) in the statistical analysis. Student t-test, Fisher's exact or Chi-sq tests were applied for unconditional statistical testing.

Results

A total of 20 outbreaks were investigated. Samples from the first 15 outbreaks have currently been examined and were included in the analyses.

Prevalence of PCV2 qPCR positive pigs was significantly different between outbreaks ($p<0.0001$).

In 5 outbreaks PCV2 was not detected by either qPCR or IHC. Six of 13 IHC positive pigs were detected in the same outbreak. The remaining 7 IHC positive pigs were evenly distributed among 4 outbreaks. Prevalence of PCV2 positive pigs were not significantly different (Chi-sq, $p>0.05$) between pigs with and without diarrhoea, table 1. Odds ratio of having diarrhoea for IHC positive compared to negative pigs were 1.67 (95% CI: 0.46 – 6.7).

Mean PCV2 excretion level in qPCR positive pigs was not significantly different ($p>0.05$) between pigs with diarrhoea (mean log10 = 6.25 copies/g faeces, sd = 1.37)) and without diarrhoea (mean log10 = 6.27 copies/g faeces, sd = 1.21).

Discussion

The non-significant differences in the prevalence and excretion levels of PCV2 in pigs with and without diarrhea imply that PCV2 was not the cause of diarrhea. Other explanations may be that the non-diarrheic pigs were in the incubation phase of diarrhea or that some pigs were able to counter the PCV2 infection without developing diarrhea.

Based on this study PCV2 should not be considered a major intestinal pathogen in weaners.

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P.051

POSTWEANING MULTISYSTEMIC WASTING SYNDROME IN CANARY BLACK PIG

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Introduction

The Canary Black Pig (CBP) is an autochthonous breed from the Canary Islands (Spain) that has been catalogued as Specially-Protected Autochthonous Breed Endangered. This is a rustic breed raised in extensive outdoor production system.

Postweaning Multisystemic Wasting Syndrome (PMWS) in pigs was first described in Western Canada (1) and then, in rest of North America and some countries of Europe (2). Porcine circovirus type II (PCV2) is considered the cause of PMWS. In 2000, it was reported the first description of PCV2 associated with porcine respiratory reproductive syndrome (PRRS) in the Canary Islands (3) in a commercial breed.

In order to establish the health status in this breed, between 2008 and 2009 the first serological study on PCV2, PRRSv and *Mycoplasma hyopneumoniae* (Mh) infection in the population of the CBP was performed. Although PCV2 antibodies were found, clinical or pathological findings associated with PMWS had not been described so far.

This work reports the first case of PMWS described in a CBP piglet of 1.5 - 2 months of age, showing cought, limb weakness, respiratory distress and finally death.

Materials and methods

A complete necropsy was performed. Tissue samples were fixed in 10% neutral buffered formalin, embedded in paraffin wax, and stained with haematoxylin and eosin (HE) for their histopathological study. Besides, immunohistochemical detection of PCV2 and PRRSv antigens was performed using available commercial monoclonal antibodies, Mab I36A and Mab 1AC7, respectively (Ingenasa, S.A., Madrid).

Results

At necropsy, the most relevant gross changes were non-collapsed lungs, interstitial edema, cranio-ventral bronchopneumonia, enlargament of lymph nodes and multiple cerebral and cerebellar petechiae.

Microscopically, a marked lymphoid depletion, with histiocytic and multinucleate giant cell infiltration and presence of multiple, grape-like intracytoplasmic inclusion bodies in macrophages were observed in lymphoid tissues. The lung showed interstitial edema, neutrophils and mononuclear cells in bronchiolar and alveolar lumina, proliferation of type II pneumocytes and multinucleate giant cells. Additionality, lymphohistiocytic inflammatory interstitial infiltrate in kidney was observed. In cerebellum, the capillary vessels displayed perivascular mononuclear infiltrate and edema.

PCV2 antigens were detected in macrophages of lung, spleen, tonsil, thymus, Peyer's patches and endothelial cells of cerebellum. PRRSv antigens were not detected in any tissue sample.

Discussion

Many studies have described the characteristic macroscopical and histopathological lesions of PMWS, but the pathogenesis of PCV2 infection is still not fully understood.

Clinical PMWS has been described in wild boards in North America and Europe, and the main lesions were observed in lymph nodes, tonsil and spleen (1,4,5). Our case represents the first description of PCV2-associated systemic infection in this breed, including brain lesions similar to those described by Correa et al., in 2006 (6).

The study of PCV2 prevalence, viral genetic characterization and concurrent infectious agents in this breed, would contribute to improve its health status, and to design conservation programs in this breed.

Acknowledgements

The authors want to thank Canary Black Pig Breeders Association and Cabildo de Gran Canaria.

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P.052
SEROLOGICAL STUDY ON PCV2, PRRS AND MH IN CANARY BLACK PIGS

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Introduction

The Canary Black Pig (CBP) is an autochthonous breed from the Canary Islands (Spain) that has been catalogued as Specially-Protected Autochthonous Breed Endangered. This is a rustic breed raised in extensive outdoor production system, although given the geographic characteristic of the island, it is in close contact with commercial herds.

Respiratory diseases in pigs occur worldwide and are present in most pig herds. They are the result of an interaction between infectious agents, the immune status of the host and the environment. Since respiratory diseases have a multifactorial aetiology, it is important to study the infectious agents simultaneously (1).

In 2009, the total number of CBP adult specimens distributed throughout the Canary Islands were about 380. In Gran Canaria, this number was not more than 149 (39.2%). In order to establish the health status in this breed, between 2008 and 2009 the first serological study on Porcine Circovirus type 2 (PCV2), Porcine Reproductive and Respiratory Syndrome virus (PRRSv) and *Mycoplasma hyopneumoniae* (Mh) infection in the population of the CBP was performed. In this communication, we report the results of this study.

Materials and methods

Samples from 73 specimens of CBP divided in 4 categories (31 sows, 6 boars, 24 piglets and 12 finishing pigs) were collected.

Antibodies against PRRS, PCV2 and Mh were measured using comercial kits: INGEZIM CIRCOVIRUS IgG/IgM (11.PCV.K2), INGEZIM PRRS UNIVERSAL (11.PRU.K1) and INGEZIM M.HYO COMPAC (11.MHY.K3).

Results

Titres of IgG antibodies against Mh were found in 18 of the 73 analysed sera (24.65%). 38 of the 73 sera (52.05%) were positive for PCV2 IgG (late infection), and 2 (2.73%), positive for PCV2 IgM (early infection). Only 1 sera (1.37%) was positive for PRRSv antibodies.

According to categories, the sows showed titres of IgG antibodies against PCV2, PRRSv and Mh; boars, piglets and finishing pigs showed titres of IgG antibodies against PCV2 and Mh. Titres of IgM antibodies against PCV2 were detected in sows and finishing pigs.

Discussion

The results of serological surveys from wild boards in Belgium (1993 and 2000), and in Spain (2000-2003) demonstrated that 33, 37 and 48% of serum were positive for PCV2 antibodies, respectively [2, 3]. In CBP, we report a seroprevalence of 52.05% and 2.73% for PCV2 IgG and IgM antibodies, respectively.

Some authors have demonstrated a seroprevalence of 21% against Mh in wild boars [4, 5]. These results are similars to those obtained in our seroprevalence study (24.65%).

A prevalence of 37.7% of antibodies against PRRSv has been demostred in wild boars [6]; however, in Spain, they have not been detected [2, 7]. In our study, we report a seroprevalence of 1.37%.

These preliminary results indicate that PCV2 and Mh are present in CBP farms and that both should be taken into account in order to prevent clinical cases. The study of respiratory diseases prevalence, genetic characterization of infectious agents and concurrent aetiologic agents in this breed, would contribute to improve its health status, and to design conservation programs in this breed.

Acknowledgements

The authors want to thank Canary Black Pig Breeders Association.

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P.053
VIDEOENDOSCOPY OF PORCINE GASTRO-DUODENAL ULCER SYNDROME (PGDUS) IN SWINES TO STUDY VIRUS – BACTERIA INTERACTION

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Introduction

To study gastrointestinal diseases in Pig farms in Venezuela, an invasive method by using videoendoscopy with a flexible fiberendoscope was standardized. Previous study early published (1) have shown that modern imaging technology is a useful tool to study Porcine Ileitis with a noninvasive techniques such as ultrasound of the abdomen of pigs with Proliferative porcine enteritis (PPE). The present protocol has proven to be a feasible, accurate and highly specific procedure to evaluate the mucosa of the gastrointestinal tract after aspirating gastro-duodenal fluids lavage (GDFL), biopsy tissues sampling for molecular biology, ultrastructure and microbiology virus-bacterial isolation, among other uses.

Materials and Methods

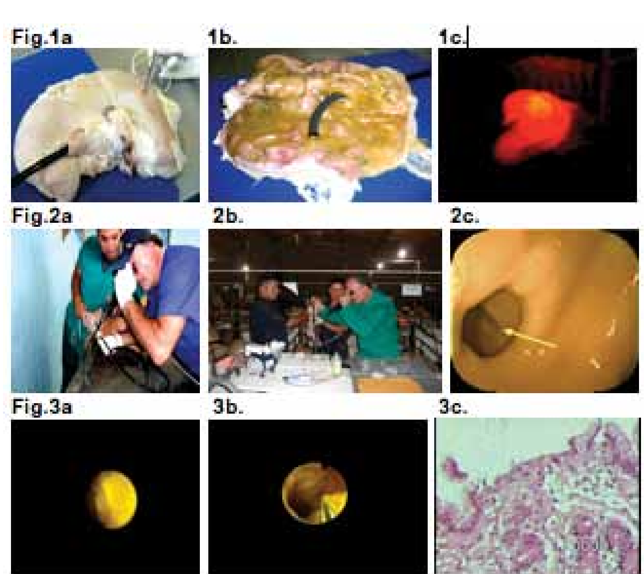
A first step study was run evaluating a total of 150 stomachs, 50 obtained from the slaughter house from fatter pigs, 50 newborn piglets studied and 50 pigs 12 week old studied at PRRS (-) PCV2 (+) farms. An exploration of the Stomach was done from each one. Those areas were videoendoscoped and handled with a flexible fiberendoscope (Eickemeyer®, Fig.1a,b,c,d), perfectly identified. Thereafter, the 50 stomachs from the slaughter were opened and the tracts of the endoscope verified (Fig.2a,b,c). Gastroduodenal fluid (GDF) was withdrawn by vaccum pump (EasyVac®). Thirty mL of GDF were collected in sterile containers for bacterial virus isolation. Paired Biopsies were taken from cardiac – fundic - pyloric and duodenal regions normal and ulcerated lesions (Fig.3a,b,Fig.2c). One for histopathology (Fig.3b,c) and the other homogenized for *Helycobacter spp*. and PCV2 DNA extraction. The prevalence of Porcine Gastro – Duodenal Ulcers Syndrome (PGDUS) was assessed. A second step study of the endoscopy protocol was done on 50 newborn-weaned pigs and 50 pigs 12 week old after 12 hours fasting (Fig.2b). The latter pigs were previously anesthetized with Xylazine (22 mgr/Kg) / Ketamine (2 mgr/Kg). After relaxation, the fiberendoscope was introduced through the mouth-pharynges and let it to be swallowed (Fig. 3c,d) through the Esophagic-Cardiac passage. Previous protection of the endoscope was done with a curve tube externally to the endoscope, mouth restraining and a dental mouth opener to protect the unit.

Results and discussion

Videoendoscopy technique proved to be very useful tool to evaluate gastrointestinal tract. Stomachs from fatter pigs showed 95% **PGDU** prevalence, 92% in weaned piglets and 20% in newborns. as well as in weaned. The degree of PGDUS ranged from mild (Grade I) to severe chronic-active (Grade IV) more frequent in fatters. Many factors are associated with the incidence of GDUS in pigs. Diet is one of them. However Table I showed the positiveness to DNA of PCV2 extracted from the GDF in the three cathegories studied. *Helicobacter spp.* DNA extractions from pigs with GDUS have been previously assessed by our research team (2). Likewise previous report by using saliva was done to assessed PCV2 DNA extraction in fatters with PND´s in deep bedding system (3). , report at the IPVS 2008, DURBAN Bermudez etal.,South Africa).

Table I. Prevalence of Porcine Gastric-Duodenal Ulcers Syndrome (GDUS) in weaned and fatters pigs.

Pigs	GDUS	DNA *Helicobacter*	PCV2/ rtPCR
Newborns = 50	20%	-	+
Weaned = 50	92%	+	+
Fatters = 50	95%	+	+



Acknowledgements

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P.054

HIGH LEVELS OF PCV2 MATERNAL ANTIBODIES FOUND IN THREE WEEK OLD PIGLETS UNDER FIELD CONDITIONS MIGHT INFLUENCE THE CHOICE OF VACCINATION PROGRAMME

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Introduction

It has been repeatedly demonstrated that Ingelvac CircoFLEX® is efficacious in the presence of high maternally derived antibodies (MDA) (2, 3). However, for another subunit piglet vaccine a possible interaction has been reported (1). In this context, a label claim extension for this product was granted, which makes it possible to apply the vaccine once only in case of low to medium levels of MDA. In case of high levels of MDA it has to be applied twice (4). Martens et al. (5) classified antibody levels > 10 log 2 as 'high'. The objective of this study was to investigate the prevalence of piglets with high levels of MDA under field conditions.

Materials and methods

In total 5 Belgian farms participated in the study. In each farm, 25 piglets originating from 5 sows (1 primipareous and 4 multipareous sows) were blood sampled at 3 weeks of age. In none of the farms sow vaccination against PCV2 was applied and the piglets were unvaccinated against PCV2 at the time of sampling. Within each litter, 5 piglets were randomly selected, but cross-fostered piglets were excluded.

The serum samples were analysed for PCV2 antibodies with the Serelisa® PCV2 Ab Mono Blocking ELISA (Synbiotics Corp). The results are expressed in log 2 titers. Samples with an antibody level below the detection limit are shown as zero.

Results

Figure 1 shows the log 2 PCV2 antibody titers of all sampled piglets in the 5 farms. Each dot represents 1 piglet and dots with the same colour and shape indicate piglets from the same farm. Table 1 indicates the percentage of piglets with 'high' (> 10 log 2) and 'low to medium' (< 10 log 2) antibody titers.

In total, 55% of the piglets showed 'high' antibody levels and piglets with 'high' antibody levels were present in each of the sampled farms (ranging from 40% in farm 5 to 88% in farm 2).

Figure 1 Distribution of piglet PCV2 antibody titers in 5 commercial farms

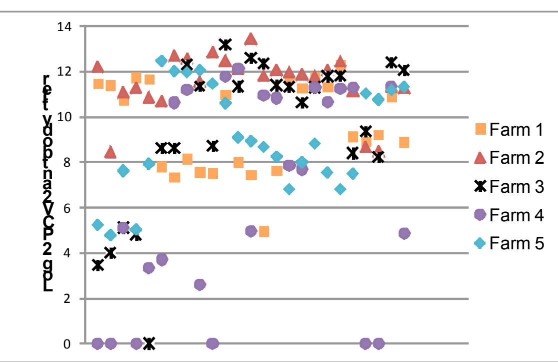

Table 1 Percentage of piglets with log 2 PCV2 antibody titers < and > 10 log 2

log 2 PCV2 antibody titer	< 10 log 2 (% of samples)	> 10 log 2 (% of samples)
Farm 1	52,0	48,0
Farm 2	12,0	88,0
Farm 3	44,0	56,0
Farm 4	56,0	44,0
Farm 5	60,0	40,0
Total	**44,8**	**55,2**

Discussion

At the age of 3 weeks, high antibody levels were commonly observed. Ingelvac CircoFLEX® can be used as a single dose in these situations as it has been shown to break through high levels of MDA (2, 3). However, the other subunit piglet vaccine should be applied twice following label recommendations. Postponing vaccination until MDA levels have dropped is not advisable. It would leave a significant number of pigs -that already have low MDA levels at 3 weeks of age- unprotected against an early PCV2 challenge.

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P.055

COMPARATIVE EFFICACY OF TWO PCV2 VACCINATION PROGRAMS IN CANADIAN PIGS

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Introduction

PCVAD problems were confirmed in a Canadian 1200 sow herd about 8 weeks post placement in finishing units. Different vaccination programs were tested over time to control the condition. In the end two programs appeared to offer a better protection and were further compared. This paper describes the results that were obtained.

Materials and methods

Pigs were either vaccinated with a full dose (2 mL) of a two-dose vaccine at weaning (about 21 days of age) and 3 weeks later, or with a full dose (1 mL) of a one-dose vaccine (Ingelvac CircoFLEX, Boehringer Ingelheim) at weaning. The performance of pigs vaccinated with these two different vaccination regimens was compared.

Results

Tables 1 & 2 show the results that were obtained in the nursery and finishing units.

Discussion

A weakness of the present study is that the batches of pigs vaccinated with the different vaccines were not raised at exactly the same time. For example when considering the nursery performance, the pigs were vaccinated with CircoFLEX from June to August 2008, and with the two-dose vaccine from September 2008 to April 2009. However the health status on the farm remained stable during that time period, and no significant disease challenges are thought to have played a role in the results obtained. Similarly no significant changes in terms of management, medication, genetics, personel or feed were done during that time. The results suggest that on that farm, the performance of pigs vaccinated with one dose of Ingelvac CircoFLEX was better than that obtained with two full doses of the two-dose vaccine.

Table 1. Nursery performance of pigs vaccinated with the two-dose vaccine or one dose of Ingelvac CircoFLEX.

	2-dose vaccine	CircoFLEX
# pigs	4185	4448
# batches	4	4
Entry wt (kg)	5.60	5.56
% mortality	2.68 [a]	1.26 [b]
FE	1.75	1.67
Weight gain, kg	25.74	27.69
Days in period	59	61
ADG, gr	440.2	452.0

a,b: $P < 0.0$

Table 2. Finishing performance of pigs vaccinated with the two-dose vaccine or one dose of Ingelvac CircoFLEX.

	2-dose vaccine	CircoFLEX
# pigs	3416	3206
# batches	6	6
Entry wt (kg)	31.4	31.3
% mortality	4.81	3.95
FE	2.90	2.90
Weight gain, kg	86.3	86.7
Days in period	108 [a]	101 [b]
ADG, gr	803 [a]	854 [b]

a,b: $P < 0.05$

P.056

THE CONTRAST BETWEEN INGELVAC® CIRCOFLEX AND TISSUE HOMOGENEATE ON PCVD CONTROL IN A 2000-HEAD SOW FARM IN CENTRAL CHINA

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Introduction

Post-weaning multisystemic wasting syndrome (*PMWS*) and other porcine circovirus type 2 related diseases (PCVD) are present in virtually all pig producing countries nowadays (1). The disease lead to severe economic losses to the Chinese pig industry since the first report in 2000 (2). No commercial and effective vaccine against PCV2 was available in China before 2010. Tissue homogenate hence was used in recent years for domestic PCVD control. The purpose of this study was to compare the effects on control of PCVD and production performances between Ingelvac® CircoFLEX and tissue homogenate.

Materials and methods

The trial was conducted on a 2000-sow farrow-to-finish farm that suffered from PCVD for years, tissue homogenate and various medication programs were tried in these years, but none of those methods made production performances return to the optimum level. Consistent fluctuation of culling and death rates in the nursery and fattening house were the main causes for economical losses. Clinical signs start in 8 to 9 week old nursery pigs, including wasting, paleness and diarrhea. Some grower-finisher pigs show typical lesions of PDNS. Gross lesions include enlarged kidneys, as well as enlarged inguinal and enteric lymph nodes. Presence of PCV2 was confirmed by PCR.

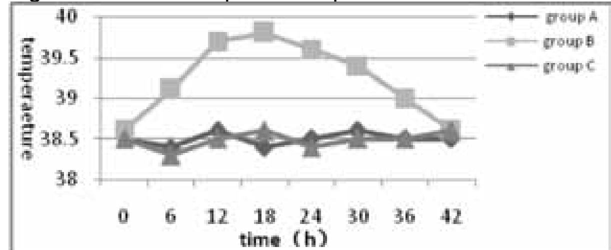
A side-by-side experiment was carried out in this farm in March 2010. A total of 600 14-day-old piglets were randomly divided into 3 groups: group A was vaccinated with 1ml Ingelvac® CircoFLEX (n=201), group B with 4ml tissue homogenate (n=201), group C (n=198) was set as control group that were treated with 1ml normal saline intramuscularly. The different groups were kept in the same house, but different pens. Each group was divided into 13 pens. Every pen was weighted at weaning (25 days of age), transfer from nursery to grow-finish (56 days of age) and before slaughter (170 days of age). Feed intake was measured by pen as well. Rectal temperatures of 20 piglets in each group were measured and recorded every 6 hours during the first 42 hours post vaccination. Culling and mortality rates (CMR), average daily weight gain (ADG) and feed conversion rate (FCR) were measured during nursery and fattening.

CMR and ADG were analyzed by LSD test while FCR was compared by Dunnett's T3 test using the PASW STATISTICS 18.

Results

Body temperatures were recorded as shown in Figure 1. The body temperatures in group A and group C kept normal, while those in group B were increased after vaccination (figure.1).

Figure 1. Rectal temperatures post vaccination



Production performances were significantly improved in group A, compared to group B and to group C. The CMR of group B was significantly lower than group C, while the FCR and ADG of group B were poorer comparing with group C (table 1).

Table 1. Production performances in 3 groups (from 25 days to 170 days of age)

	Group A	Group B	Group C
CMR(%)	2.47±1.15[a]	9.65±1.94[b]	16.19±2.84[c]
ADG(g/d)	715.72±2.63[a]	628.6±7.91[e]	662.09±4.99[c]
FCR	2.502±0.007[a]	2.904±0.039[e]	2.715±0.015[c]

Different superscript letters in the same row indicate significant differences.[a,b]: $p<0.05$, [b,c]: $p<0.05$, [a,c,e]: $p<0.01$

Discussion

Ingelvac® CircoFLEX not only effectively reduced CMR and FCR, but also increased ADG, demonstrating excellent and superior efficacy, while tissue homogenate caused high fever stress and slow growth.

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P.057

ASSESSMENT OF REPRODUCTIVE PERFORMANCE IN A MEXICAN HERD VACCINATED WITH INGELVAC CIRCOFLEX®

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Introduction

Porcine Circovirus type 2 (PCV2) is commonly associated with retarded growth and increased mortality in the grow-finishing phase. Porcine circovirus associated disease (PCVAD) includes pneumonia, systemic infections, lymphadenopathy with lymphoid depletion, enteritis and nephritis. Other presentations of PCVAD include post-weaning multisystemic wasting syndrome (PMWS) and porcine dermatitis and nephropathy syndrome (PDNS). Less common are reproductive losses attributed to infection by PCV2. The clinical signs are described as a 50% increase in late term abortions, mummified fetuses, stillborn piglets and weak, non-viable piglets at birth. The cases of reproductive diseases related to PCV2 have been described in new herd populations and in new sources of replacements. The first reported case of reproductive failure related to PCV2 in sows was in 1999. That case described PCV2 as the causal agent after it was isolated from a litter of aborted piglets from a farm experiencing late-term abortions and farrowings with a greater incidence of stillborn and mummified piglets[1]. No association was found with other pathogens causing abortions, such as Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), Porcine Parvovirus or Leptospira interrogans. This abstract describes the reproductive performance before and after implementing PCV2 vaccination in sows.

Materials and methods

This is a before/after comparison study in a PRRS negative, 1,200 sow herd, which started to vaccinate pigs and sows with Ingelvac® CircoFLEX from the middle of June 2009. The decision to start vaccinating the herd against PCV2 was due to 2 factors: a noticeable increase in mummies and stillborns was observed with no association with other co-factors; and growing piglets showed clinical symptoms of PCVAD with viral loads averaging 9.67 logs. The vaccination protocol implemented was a 1 ml dose given to sows between 5 and 2 weeks before farrowing, replacement females at 27 weeks of age, and piglets at weaning, approximately 3 weeks of age.

Results

Differences were observed comparing the reproductive performance before and after PCV2 vaccination. There was an increase in the percentage of live born piglets (figure 1), and a reduction in percentage of stillborn piglets, mummies and in pre- weaning mortality (figure 2). However, no tissue samples were taken of stillborn and mummified piglets thus no confirmatory immunohistochemistry was performed. Even so, the relationship between PCV2 and clinical reproductive failure of the non-vaccinated animals is evident.

Figure 1: Control chart showing % of piglets born alive before and after vaccination

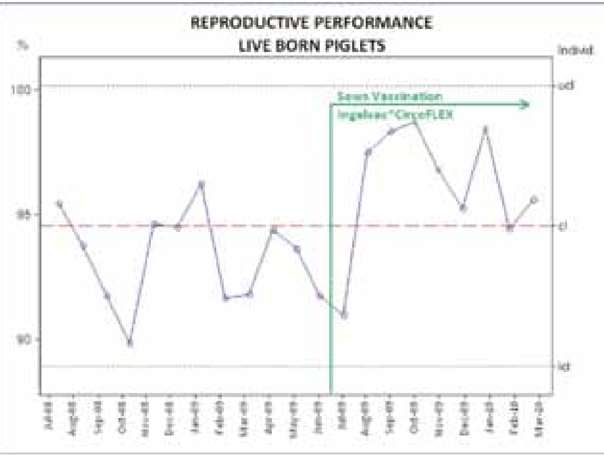
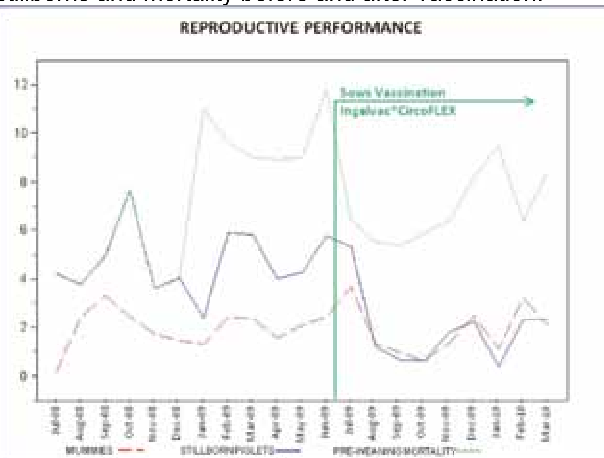


Figure 2: Control chart showing % pre-weaning mortality, stillborns and mortality before and after vaccination.



Discussion

In Mexico we still need to do more research on reproductive failure associated with PCV2. This monitoring shows that there are clear improvements in the reproductive parameters which may be attributed to the use of PCV2 vaccine

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P.058

THE IMPACT OF PCV2 VIREMIA IN A HIGH HEALTH CANADIAN SWINE HERD, A VACCINATION TRIAL COMPARING TWO COMMERCIAL VACCINES

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Introduction

Subclinical infections with PCV2 are common[1,2] and can occur in the absence of co-factors or if vaccination does not prevent viral replication[3]. PCV2 viremia produces immune system activation[4] which causes the redirection of nutrients intended for growth to counteract disease challenge[5]. The objective of this field trial was to compare the productivity of nursery and grower-finisher (G-F) pigs vaccinated with a labeled dose of one of two commercial circovirus vaccines or a placebo. Productivity was measured as average daily gain (ADG), feed disappearance, and mortality.

Materials and methods

A total of 2,146 pigs were selected from a PRRS- and *M. hyopneumoniae*-free herd. The pigs were assigned to one of three treatment groups: 1. One dose (1-D) pigs vaccinated with Circoflex® BIVI (n=1026); 2. Two dose (2-D) pigs vaccinated with Circumvent® ISPAH (n=1020); and 3. Controls injected with saline (n=100). Pigs were individually weighed at approximately 3, 11 and 19 weeks of age as well as prior to slaughter (at least 107 kg). Blood samples were taken from a random sample of 122 pigs at approximately 3, 9, 15, 19, and 23 weeks of age and also during the final week of shipping. Viremia was measured using qPCR.

Results

Throughout the entire G-F phase, the ADG of vaccinated animals outperformed that of unvaccinated controls ($p<0.01$). Also during this time, controls had a higher mortality (5.1%) than 1-D (1.7%) and 2-D (1.6%) pigs ($p<0.05$). In the grower phase (9 – 15 wks) the ADG of the one-dose and two-dose vaccine groups during Period 2 did not differ ($P > .05$) However, the ADGs of the one-dose and two-dose vaccinated pigs were higher than the ADG of control pigs. After controlling for start weight and weaning cohort, ADG was 34.1 g per day higher for the one-dose group ($P < .01$) and 34.8 g per day higher for the two-dose group compared to controls ($P < .01$).

While the control group had a higher percentage of viremic pigs during the G-F phase compared to the vaccine groups ($p<0.01$), the 1-D vaccinated pigs were also more likely to be viremic compared to the 2-D vaccinated pigs ($p<0.01$).

In the second half of the G-F phase, when qPCR results indicated the highest natural PCV2 challenge, the ADG of the 1-D pigs decreased. In the grower phase, the ADG of 1-D pigs was 892 g/day whereas in the finisher phase it was 857 g/day. From 19 weeks of age to slaughter, the 1-D vaccinates had an

ADG that was 42.3 g/day lower than the 2-D vaccinates ($p<0.01$). Furthermore, the coefficient of variation was higher in the one-dose vaccine pigs (27.5%) than the two-dose vaccine pigs (19.3%).In addition, the 1-D vaccinates had a higher amount of feed disappearance compared to the 2-D vaccinates throughout the G-F phase. This means that, although the 1-D pigs were growing at a slower rate, they were potentially eating more feed than the 2-D pigs.

Discussion

Compared to the non-vaccinated control pigs, vaccination reduced mortality and increased ADG. However, only the 2-D vaccine was able to control viremia and maximize ADG during the high PCV2 challenge in the finisher phase. The 1-D vaccine failed to protect the pigs from 19 weeks of age to market. During this time the pigs exhibited viremia and a drop in ADG.

Acknowledgements

The swine group of Clinical Research at the U of G. Producers and veterinarians. Dr Brad Thacker, Technical services Intervet Inc.

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P.059

EXPERIENCE WITH INGELVAC CIRCOFLEX® ON FIVE PIGS FARMS IN RUSSIA

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Introduction

Porcine circovirus disease (PCVD) is wide spread in Russian pig farms (1, 2). Ingelvac CircoFLEX® became available in Russia in June 2010. This paper summarizes the first efficacy results available from Russia.

Materials and methods

The field study included five farms situated in different parts of country. Farm A is multisite, farms B-E are one-site, farrow-to-finish farms. All farms were PCV-2 positive (in ELISA and PCR) and had PCVD history, like PRDC or PMWS, before vaccination (table 1). Farms also were positive to PRRSV (A, C, E), *Mycoplasma hyopneumoniae* (A, E), *APP* (A, B, C, E).

In farms A, B, C, D 3 week-old pigs were vaccinated, while in farm E pigs were vaccinated between 15 to 52 days of age (mass vaccination) with a single dose (1ml) of Ingelvac CircoFLEX®. Non-vaccinated (NV) control groups were kept in same conditions as vaccinated pigs (side-by-side), farm B-E in different pens in the same barn, on farm A in different barns on the same site. Nursery performance was evaluated in 5 farms (A, B, C, D, E), while grow-finish performance data is so far available from 3 farms (A, B, D).

Table 1. Short characteristics of study farms before PCV-2 vaccination.

Farm	Sows	Main problems	
		nursery site	finishing site
A	12,000	no	PRDC
B	2,650	PRDC	PRDC
C	1,600	PMWS, PRDC	PRDC
D	530	PMWS	PMWS
E	5,100	PMWS, PRDC	PRDC

Results

Mortality and cull in nursery and growing-finishing sites reduced after vaccination. Livability increased 2.9-5.6% for nursery and 1.08-4.21% for growing-finishing sites.

Average daily gain (ADG) improved by 8.3-32.3 g/day in the nursery and 7.0-73.3 g/day in growing-finishing sites. In farms B and C, antibiotic use was reported as well, and costs for antibiotics were reduced by 27.3-29.3%. Return on investment (ROI) was 2.58:1, 8.54:1 and 5.01:1 for farms A, B, D in this study, correspondingly.

Table 2. Performance results in nursery site.

Farm	Group	Mortality, %	Cull, %	Livability, %	ADG, g/day
A	CircoFLEX, 9526 pigs	1.39	0.43	98.18	404.3
	NV control, 12133 pigs	1.43	0.36	98.21	391.3
B	CircoFLEX, 1050 pigs	2.57	1.24	96.19	418
	NV control, 1050 pigs	5.33	4.0	90.67	385.7
C	CircoFLEX, 1917 pigs	3.50	0.20	96.30	n.d.
	NV control, 1063 pigs	18.35	2.35	79.30	n.d.
D	CircoFLEX, 678 pigs	2.06	0	97.94	528.3
	NV control, 745 pigs	4.97	0	95.03	520.0
E	CircoFLEX, 12059 pigs	10.55	4.61	84.84	353.5
	NV control, 2613 pigs	13.62	7.12	79.26	335.8

n.d. – no data available

Table 3. Performance results in growing-finishing.

Farm	Group	Mortality, %	Cull, %	Livability, %	ADG, g/day
A	CircoFLEX, 4766 pigs	1.24	1.43	97.33	819.8
	NV control, 11142 pigs	1.62	2.13	96.25	784.2
B	CircoFLEX, 1010 pigs	1.58	0.30	98.12	880.0
	NV control, 952 pigs	4.52	1.57	93.91	806.7
D	CircoFLEX, 664 pigs	1.05	0	98.95	865.3
	NV control, 708 pigs	2.40	0	97.60	858.3

Discussion

The results of this study demonstrate the good efficacy of Ingelvac CircoFLEX in Russian pigs. On all farms the performance was improved in the vaccinated pigs, though the extend of improvement differed depending on the specific farm situation.

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P.060

APPLICATION OF A STATISTICAL APPROACH TO OPERATIONAL DATA FROM PIG HERDS AND TO PERFORMANCE RATING BY VETERINARIANS AND PIG HOLDERS FOR DETERMINATION OF THE EFFICACY OF INGELVAC CIRCOFLEX®

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[(1)] University of Veterinary Medicine Hannover, Germany; [(2)] Boehringer Ingelheim Pharma GmbH & Co. KG ; [(3)] Boehringer Ingelheim Vetmedica GmbH ; [(4)] Boehringer Ingelheim Animal Health GmbH .

Introduction

In a project based on a telephone survey among German farmers and vets it was demonstrated that this method combined with standardised questionnaires is an efficient tool to investigate application methods of new vaccines and customer satisfaction on a broad basis. Data assessed in this survey regarding overall satisfaction with Ingelvac CircoFLEX® (Boehringer Ingelheim) were in accordance to conclusions drawn on various studies performed in single herds (1, 2). However, due to unexpected almost unanimous positive response, further analyses of factors potentially influencing the outcome requested a complex statistical approach. The aim of this study was to utilize principal components analyses, cluster analyses and other multivariate tests for this purpose.

Materials and methods

Datasets from 227 herds that currently applied or have applied the PCV2 vaccine to pigs were included in this study. Since 1-, 2- and 3-site production systems were surveyed, the herds were allocated in one of two subsets, where only applicable variables were analysed. Group 1 was comprised by herds with suckling and nursery pigs, whereas herds in group 2 housed no suckling pigs, optionally nursery pigs and definitely fattening pigs. Overall 12 variables evaluating the subjective satisfaction with the vaccine were comingled to an abstract Y-variable (depended variable for further models), which was characterized by a binary outcome: good/excellent satisfaction (green cluster) and moderate satisfaction (red cluster). The other 168 variables comprised by various information about diagnostics, vaccination, housing, management, were considered as X-variables (independent variables). Data analysis was performed using the SAS® for Windows v.8.2.

Results

The abstract level of satisfaction with the PCV2 vaccine was determined for both groups, resulting in allocating 106 (G1) and 64 herds (G2) into the green cluster. The remaining 46 (G1) and 35 herds (G2) were allocated into the red cluster. PCA biplots (Figure 1) were used for descriptive purpose to select those subjective responses for further analyses describing most efficiently the variance within the outcome of X-variables. Univariate analyses included Fischer´s exact test and calculation of odds ratios (Table 1). The final multivariate model for each group included parameters automatically selected by stepwise logistic regression analysis. In group 1, herds using the vaccine due to PCV2 related health problems had a 2.4-fold increased chance (1/OR) belonging to the green cluster. A similar increase (2.5-fold) was observed in herds supplementing iron per injection only once instead of twice in piglets. In the final model for group 1, the diagnosis of other diseases than PCV2, the reason for vaccine administration being not another than PCVAD and the single injection of iron had significant influence on allocating into the green cluster (P<0.05). In group 2, only unchanged time or delay of time of vaccination influenced the satisfaction (P<0.05).

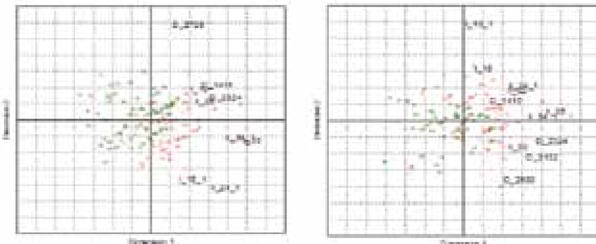

Figure 1. PCA biplots with response clustering for group 1 & 2 (green and red figures indicate herds; blue characters represent the subjective variables)

Table 1. Odds ratios and confidence intervals of X-variables (outcome differentiated according to cluster in 2x2 table)

X-variable	Group 1		Group 2	
	OR	LCL/UCL (95%)	OR	LCL/UCL (95%)
Reason for using CircoFLEX: NOT wasting, mortality or PDNS, BUT pneumonia, diarrhoea or marketing (vets´ answer)	1.41	0.588/3.358	1.93	0.772/4.873
Reason for using CircoFLEX: NOT wasting, mortality or PDNS, BUT pneumonia, diarrhoea or marketing (farmers´ answer)	**0.41**	0.168/0.983	0.76	0.306/1.911
Diagnosis of other diseases than PCAD in the herd	0.47	0.195/1.121	0.94	0.362/2.485
Vaccination of sows against PRRS	0.69	0.270/1.762		
Vaccination of gilts against PRRS	0.57	0.196/1.632		
Applying once or twice iron per injection to the suckling pigs	**2.52**	1.035/6.170		
Current use of CircoFLEX in the herd (vets´ answer)			0,34	0.028/3.199
Current use of CircoFLEX in the herd (farmers´ answer)			0.26	0.004/5.279

Discussion

The statistical approach used in this study was feasible to scientifically assess “satisfaction”, and to determine factors influencing farmers´ and vets´ opinion about safety and efficacy of a new vaccine.

Acknowledgements

The project was supported by Boehringer Ingelheim.

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P.061
EFFICACY OF INGELVAC CIRCOFLEX® IN ONE SITE LARGE-SCALE FARM WITH SERIOUS PRDC OUTBREAKS

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Introduction

Today PCV2 is regarded as one of the main pathogens involved in the porcine respiratory disease complex (PRDC). The efficacy of Ingelvac CircoFLEX® in PRDC affected farms has been shown earlier (1). In Russia Ingelvac CircoFLEX® became available mid 2010. The aim of our study was to analyse the efficacy of Ingelvac CircoFLEX® on a large-scale Russian farm with serious PRDC outbreaks in nursery and fattening periods.

Materials and methods

The farrow-to-finish large-scale study farm (2,650 sows) was situated in the North-West of Russia. The first serious PRDC outbreaks started in spring 2009 (nursery: day 50-55, finishing: day 110-150). Postmortem examinations of finishing pigs revealed APP-like lesions. *Salmonella choleraesuis*, *Haemophilus parasuis*, *P. multocida (P.m.)* and APP (no typification) were isolated from nursery and finishing pigs. Based on the diagnostic results a vaccination programme against APP and *P.m.* was started. Pigs were vaccinated twice with a commercial inactivated vaccine against APP (serotypes 2 and 6) and *P.m.* type D (table 1). Performance results before and after APP and *P.m.* vaccination are summarized in table 1.

Table 1. Herd parameters one year before and one year after start of APP and *P. m.* vaccination programme.

Item	Before		After	
	Nursery	Finishing	Nursery	Finishing
Mortality,%	5.88	6.80	5.87	3.50
Cull, %	6.04	1.33	2.26	2.49
Livability, %	88.08	91.87	91.87	94.01
ADG, g	375.0± 5.1	780.45 ±3.25	385.7 ±4.3	808.7 ±4.25

Though the health situation improved clinical signs related to PRDC were still observed in grow-finishing pigs. At that time a trial was initiated. In total 2,100 pigs were allocated to two treatment groups. Half of the pigs received one dose (1ml) of Ingelvac CircoFLEX® vaccine at 3 weeks of age, while the other half was vaccinated -as previously- against APP and *P.m*. Treatment and control groups were kept in different pens in the same barns.

Results

Performance results during the trial are summarized in table 2. PRDC signs and antibiotics costs were considerably reduced among PCV-2 vaccinated pigs. Return on investment (ROI) was calculated as 8.54:1.

Table 2. Performance results in nursery and growing-finishing pigs (Mean±SD).

	Nursery		Finishing	
	Circo FLEX	Control	Circo FLEX	Control
No. of pens	3	3	3	3
No. of pigs	1,050	1,050	1,010	952
Mortality, %	2.57	5.33	1.58	4.52
Cull, %	1.24	4.0	0.30	1.57
Transferred to hospital pen,%	5.62	8.0	0	0
Livability, %	96.19	90.67	98.12	93.91
ADG, g	418.0 ±3.0	385.7 ±4.0	880.0 ±10.0	806.67 ±5.77
Mean starting weight, kg/pig	8.17 ±0.40	7.87 ±0.31	27.60 ±0.62	25.21 ±0.27
Mean final weight, kg/pig	27.60±0.62	25.21±0.27	110.0±5.0	100.0 ±0.0
Total of weight gain, kg/pig	19.43 ±0.31	17.35 ±0.18	82.40 ±5.19	74.79 ±0.27
Term of keeping, days	72.0± 0.0	72.0± 0.0	90.0 ±0.0	97.3 ±0.6

Discussion

The better performance in the PCV-2 vaccinated animals indicate that at the time of the trial the main pathogen responsible for the clinical signs associated with PRDC was PCV-2. Pathogenic APP and P.m. might not have been present during the trial. This field trial shows that PCV-2 is an important respiratory pathogen contributing to PRDC and that Ingelvac CircoFLEX® is an effective tool to control PCV-2 associated respiratory symptoms. Similar results have been described earlier (1, 2).

Referentes

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P.062

IMPROVEMENT IN PORK CARCASS GRADE AFTER THE USE OF INGELVAC CIRCOFLEX® IN A KOREAN SWINE PRODUCTION SYSTEM

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Introduction

Since the launching of PCV2 vaccine in 2008, the Korean pork industry experienced a remarkable change. A regional evaluation conducted in 2008 and 2009 showed the efficacy of PCV2 vaccination (Ingelvac CircoFLEX®) in reducing mortality rate (1). Carcass quality is an economically valuable measure of swine productivity. Dodram Pig Farmer's Cooperative, one of the major pig operations in Korea, wanted to evaluate the effect of vaccination with Ingelvac CircoFLEX® on pork carcass grade as an internal measure of farm productivity. The objective of this study was to demonstrate that vaccination with Ingelvac CircoFLEX® will improve the pork carcass grade in a large Korean pig cooperative.

Materials and methods

Ingelvac CircoFLEX® was introduced into Dodram Pig Farmer's Cooperative's vaccination protocol. A before/after comparison was made to compare pork carcass grade. 6 farms (farrow-to-finish or 2 site) in Gyeonggi, Chungcheong, Jeonla, and Gyeongsang province were selected. These farms represent 2200 sows ranging from 280 to 500 sows each. The farms selected for this study were considered to be PRRS stable during the time of the study. All pigs in this study were fed the same diets supplied from Dodram Cooperative Feed during the entire period.

The 6 farms all had either early or late PCVAD issues during the fattening period. All farms vaccinated 3 week old piglets with Ingelvac CircoFLEX® (1 ml dose). No PRRS vaccination was used.

The survey covered a period of 24 months between 2007 and 2010 (12 months before and 12 months after vaccination). The pork carcass grade was compared before and after vaccination with Ingelvac CircoFLEX®. 28,000 pigs were graded before vaccination and 33,800 pigs after vaccination.

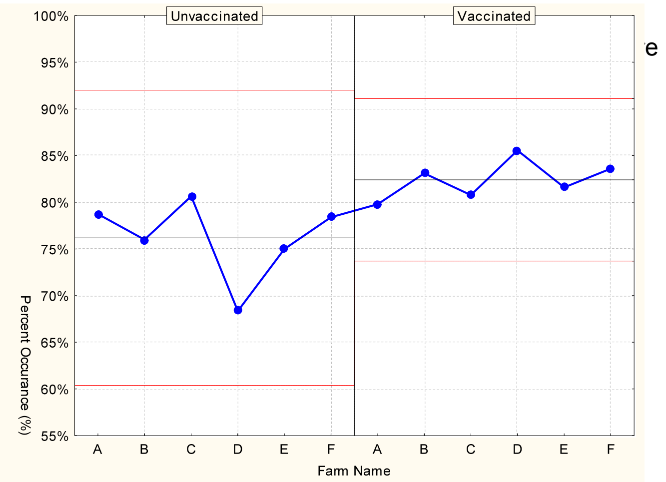
All the pork carcasses in Korea are graded by KAPE (Korea Institute for Animal Products Quality Evaluation) both in conformation (grade of A,B,C and D on carcass weight, back fat thickness, lean percent etc.) and quality (grade of 1^{+},1,2 and 3 composed of marbling, lean color, conditions of belly streaks). Percentage of carcasses with Conformation Grade A&B and with Quality Grade 1^{+}&1 were compared. These values bring the highest premium price at slaughter.

Results

Table 1: Average percentage of pork carcass grade before and after Ingelvac CircoFLEX® vaccination

	Before vac.	After vac.	Improvement
Conformation Grade A & B (ave. %)	76.2%	82.4%	**+6.2%**
Quality Grade 1^{+} & 1 (ave. %)	73.0%	82.4%	**+9.4%**

In the selected farms there was a 6.2% improvement in Conformation Grade and a 9.4% improvement in Quality Grade. The percentage of pork carcass grade for each farm was averaged for the 12 month period before and after vaccination.



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Discussion

This study conducted by Dodram Pig Farmer's Cooperative shows that Ingelvac CircoFLEX® provides efficient control of PCVAD and has a positive effect on the improvement of the pork carcass grade. Carcass quality is a valuable measure of productivity and may become an important parameter for the Korean swine industry. This comparison demonstrated that vaccination with Ingelvac CircoFLEX® may improve pork carcass grade which leads to an increase of sale price and profit to the Korean pig producer.

References

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P.063

REDUCTION OF MORTALITY AND CULLING BY THE USE OF PORCILIS® PCV AND / OR PORCILIS® GLAESSER

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Introduction

The objective of this study was to assess the efficacy of Porcilis® PCV and / or Porcilis® Glaesser (Intervet Schering Plough AH, Boxmeer, The Netherlands) in reducing mortality and culling. The study was performed at a large pig breeding and fattening farm in Russia with a high number of losses mainly in the nursery. Clinical signs and high mortality rates indicated an involvement of PCV2, in spite of other infectious agents detected on the farm. Additionally, polyserositis was a common finding at necropsy.

Materials and methods

A total of 15 638 suckling piglets were divided in 8 groups (table 1). Piglets were assigned to one of four treatments. Group A vac was vaccinated with Porcilis® PCV, group B vac with Porcilis® PCV as well as Porcilis® Glaesser and group C vac was vaccinated with Porcilis® Glaesser. The vaccines were administered as a two-shot vaccination on the 14th and 35th day of live. In the group with combined vaccination (group B vac), drugs were administered at separate sides of the neck. Piglets of the five remaining groups served as non-vaccinated controls (table 1). Letters A-D represented subsequent batches. In batch A-C pigs were splitted in vaccinated and non-vaccinated animals. Pigs from batch D were splitted in two groups to show the degree of variation within non-vaccinated pigs. Mortality rates and number of cullings were documented until slaughter.

Table 1: Number of animals (n) and vaccination of the study groups

Group	Vaccination	n
A vac	Porcilis® PCV	1 913
A non-vac	no vaccination	1 902
B vac	Porcilis® PCV / Porcilis® Glaesser	1 883
B non-vac	no vaccination	1 965
C vac	Porcilis® Glaesser	1 911
C non-vac	no vaccination	2 069
D non-vac 1	no vaccination	1 892
D non-vac 2	no vaccination	2 103

Results

Figure 1 is showing the total losses segmented in mortality rates and number of cullings. The total losses of batch D were summed up, because results of both groups did not differ significantly. Between non-vaccinated pigs of batch D and pigs of group A vac as well as group B vac a significant difference in total losses of 18.0 % ($p \leq 0.002$) and 16.8 % ($p = 0.002$), respectively, was shown in favour of the vaccinated animals. Furthermore significant differences could be determined between group A vac and group B vac when compared to group C vac ($p = 0.001$). The losses of group C vac and D non-vac were comparable within the range of the common variation of this farm. Within the batches A-C the total losses of the non-vaccinated animals where higher than those of the vaccinated ones showing significant differences in groups with pigs vaccinated against PCV2 (A: 16.4 %, $p \leq 0.001$; B: 13.4 %, $p = 0.020$; C: 0.2 %, $p = 0.290$).

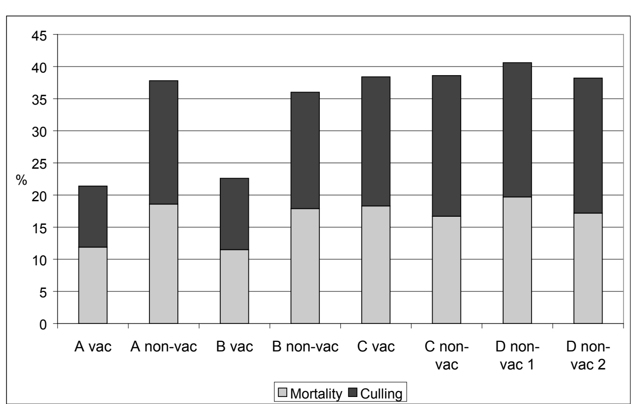

Figure 1: Mortality rates and number of cullings in the different groups

Discussion

Vaccination with Porcilis® PCV and combined vaccination with Porcilis® PCV / Porcilis® Glaesser significantly reduced the total losses during this study. This indicates that Porcilis Glaesser does not negatively interfere with Porcilis PCV. The fact that vaccination with Porcilis Glaesser had no beneficial effect suggests that during the time of study conduction *Haemophilus parasuis* infections were not major problems on the farm. In conclusion vaccination against PCV2 had a significant influence on the number of animals that died or had to be killed on this farm. This is in line with several other trials where mortality was significantly reduced after vaccination against PCV2 (1, 2, 3). Losses after vaccination were still relatively high. Other infectious causes or management faults have to be considered on farm level.

References

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P.064
EVALUATION OF THE ECONOMIC RETURN OF TWO VACCINATION PROGRAMS ON A HIGH HEALTH CANADIAN HERD

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Introduction

Immunization against PCV2 has been demonstrated to be effective for controlling PCVAD in the field. However, there are differences among products and protocols with regard to controlling viremia in vaccinated pigs. Viremia has been associated with the sub clinical infection and impaired growth rate. The objective of this study was to evaluate the economic return of Circumvent PCV vaccination (2D) in a high health Canadian herd, in comparison to pigs vaccinated with a one dose commercial vaccine (1D).

Materials and methods

Close out information from a vaccination trial published previously (Reindl et al., 2010) was used to calculate the production cost differences, variation in growth rate (CV), average daily gain (ADG) and feed disappearance and conversion (FC). Economic impact calculations were made using feed intake, pig weight sold and the average feed price at the time of the trial.

A quintile analysis was used to calculate the variation in growth rates. To predict heavier slaughter weights, mathematical models were created from the regression models to predict heavier slaughter weights (representative of US market weights).

Results

The pigs remained free of cofactor diseases during the trial (SIV, PRRS, and Mycoplasma). Differences in feed disappearance, FC and ADG varied among treatments. Pigs vaccinated with 1D and consumed 5.87 kg more of feed during the grow-finish period compared to pigs vaccinated with 2D pigs. FC was 0.1 higher for the 1D group, representing a production cost increase of $2.00/ pig, based on feed prices during the trial (Winter 2008). The ADG of the 2D vaccine group was 42.3 g per day higher than that of the 1D (P<0.01). Furthermore, the CV was 8 % higher in the 1D vaccinated pigs (27.5%) than the 2D vaccinated pigs (19.3%). Analysis of growth categories based on quintile cut-offs (very slow, slow, average, fast and very fast growing pigs) indicated that 1D pigs were growing more slowly than 2D pigs in all categories (P<.01) except the very fast pigs (the fastest 20%) where there was no significant difference (P>0.05).

Data extrapolation to heavier finisher weights predicted the same trend; 1D pigs would be expected to grow more slowly than 2D pigs.

Discussion

Even in the absence of cofactors there were significant economic losses incurred by PCV2 infection. Although clinically healthy, sub clinically affected pigs redirected nutrients intended for growth towards fighting the disease, causing variation in growth and poor feed conversion.

References

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P.065

EVALUATION OF INGELVAC CIRCOFLEX® EFFICACY IN CONTROLLING PCVD IN A PIG HERD IN MALAYSIA

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Introduction

In Malaysia, PCV2 was first isolated in 2004 and the condition was described in 2007 (1). The objective of this study is to determine the efficacy of vaccinating piglets on 21 days age with Ingelvac CircoFLEX® in a pig herd in Malaysia with severe losses due to PCV2.

Material and Methods

A farrow-to-finish farm, comprising 500 sows, with a previous history of PCVD was selected for the study. These farms reported having high mortality between 20-30% from weaning to slaughter. Herd have been diagnosed with PCVD before vaccination by necropsies in 45 day old pigs. A total of 500 piglets were selected and divided into vaccinated and non-vaccinated groups, 250 piglets each. All piglets in the vaccinated group were vaccinated with Ingelvac CircoFLEX® (1 ml) intramuscularly at 21 days of age.

Both groups of piglets were reared in separate pens in the same house. Pigs were weaned at about 25 days of age. Body weights and mortality were recorded at the time of weaning, 75 days of age (transfer to grow-finish unit), and before slaughtering. Through out the study, pigs from both groups with poor body condition, having respiratory distress or no respond to treatment were culled.

Presence of skin lesions in both groups was recorded. Mortality and culls were statistically compared between the groups using Chi-square test.

Results

A significant reduction of 55% in the total losses (mortality and culls) was observed in the vaccinated group. In this group the mortality and cull was 10.8% compared to 24 % in the non-vaccinated group ($p<0.05$). The difference in ADG (Average Daily Weight gain) at the age of 75 days was 3g/day. ADG differences become obvious after day 75 until slaughtering. The difference in ADG in this period reaches 38g/day. The wean-to-slaughter ADG of vaccinated group is 21g/day higher. Performance results are summarized in Table 1.

Thirty per cent of the pigs in the non-vaccinated group were reported to have skin lesions after day 80 of age, while no skin lesion was reported in the vaccinated group until slaughtering.

Table 1: Performance of vaccinated and non-vaccinated pigs.

	Vaccinated	Non-vaccinated	Difference
No. of piglets	250	250	-
Mortality % (wean-slaughter)	5.2	11.2*	-6 (-54%)
Culls % (wean-slaughter)	5.6	12.8*	-5.6 (-56%)
ADG g/day (wean - day 75)	373	370	+3
ADG g/day (day 75 -slaughter)	768	730	+38
ADG g/day (wean - slaughter)	538	517	+21

*Statistically significant between the groups ($p<0.05$)

Dicussion

Although the farm had a high incidence of other disease, including *Haemophilus parasuis*, mycoplasmosis and *Actinobacillus pleuropneumoniae*, mortality and culls were reduced significantly. From the results of the study, vaccination against PCVD with Ingelvac CircoFLEX® reduced PCVD related mortality throughout pig's life and improved growth performance in this Malaysian pig farm.

Acknowledgements

This study was supported by Mr.Goh Swee Jin. General Manager and farm supervisor of Tai Lee farm, Johore.

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P.066
PCV2 VACCINATION AS INSURANCE: EVEN MILD PCV2 CAN HIT HARD IN SOME BATCHES

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Introduction

Despite the fact that PCV2 virus can have devastating influence on a pig herd, pig herds can also be mildly infected by PCV2 virus without obvious clinical signs of PCV2 infection.

In such herds, it can be difficult to visualize the benefits of PCV2 vaccination. Batch variation might interfere with evaluation of a vaccination programme, and a side-by-side (SBS) trial will often be necessary to fully explore the difference between PCV2-vaccinated and non-vaccinated pigs.

The present study was carried out to evaluate the effect of PCV2 vaccination in a Danish SPF herd, where PCV2 virus was detectable in blood samples from a few pigs in some batches.

Materials and methods

The study herd was a wean to finish herd receiving batches of approx. 250 pigs every 2nd week. The study was designed as a double blinded SBS trial in 3 batches and included 2 groups of pigs: One group vaccinated with Ingelvac CircoFLEX® (Boehringer Ingelheim, 1 ml i.m.) at 2 weeks of age, and one non-vaccinated group. The study included 768 pigs.

The pigs were weaned and moved to the study herd at 4 weeks of age. Treatment groups were kept in different pens in the same barn, and the evaluation was blinded to the treatment groups.

The number of dead pigs, pigs moved to the disease unit and pigs too small for delivery when the barn was emptied (approx. 19 weeks of age) was summed up as losses. After slaughter of 50% of the pigs in a batch (approx. 17 weeks of age), the remaining pigs was counted.

The pigs were weighed at weaning, 6 and 14 weeks after weaning. ADWG was calculated from weaning to 14 weeks of age. Statistical comparison of the groups was done with chi-square test, Students t-test or Mann-Whitneys U-test with p=0.05 as level of significance. The economic calculation was done using key values from the Danish Pig Producers organisation (1).

Results

Overall, vaccination gave and a significant increase of 22 g/day in ADWG from weaning until 14 weeks of age (p=0.0126) and a 50% reduction of the losses (p=0.0326) (table 1). The number days to slaughter was reduced by 1.5 day (p=0.0221) with the same slaughter weight in both groups. The economic value of the reduced losses alone gave a ROI of 1:1.7.

Table 1. PCV2 vacc. compared to non-vacc. pigs.

Batch	Grp.	# pigs			ADWG[b]
		Total	Lost	Present at 17 w[a]	g/day
1	Vacc	132	3*	39	806
	Ctrl	132	12	34	791
2	Vacc	126	5	39*	832
	Ctrl	126	4	57	820
3	Vacc	126	5	39	787*
	Ctrl	126	10	45	741
All	Vacc	384	13*	117	806*
	Ctrl	384	26	136	784

* = Statistically significant difference between groups ($p<0.05$). [a] = No. of pigs present in the barn at the time when 50% of the batch was slaughtered (about 17 weeks of age), [b] = Mean ADWG from weaning to 14 weeks of age.

The largest difference in the production parameters was seen in the 3rd batch, where PCV2 vaccination increased weight 14 weeks post weaning by 5 kg ($p<0.0001$). Days to slaughter of that batch are shown in figure 1.

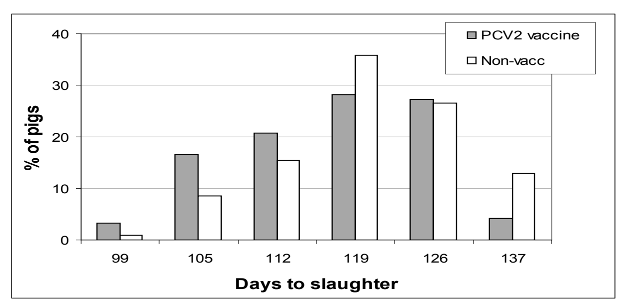

Figure 1. Days to slaughter for batch No. 3 (252 pigs)

Discussion

Even in this herd with a very low level of infection with PCV2, all batches got a benefit of vaccination. The magnitude of the benefits varied between batches. Still, the effect in the 3rd batch was large enough to justify a vaccination programme for all 3 batches.

Hence, PCV2 vaccination can be seen as insurance: Cost benefit calculation on a single batch might not always give a positive result, but in the long run, PCV2 vaccination of pigs clearly pays off.

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P.067

A FIELD STUDY TO DETERMINE THE SAFETY AND EFFICACY OF PORCILIS®PCV UPON SIMULTANEOUS AND CONCURRENT USE WITH PORCILIS®M HYO IN A FARM WITH LATE PCV2 INFECTION

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Introduction

Within the last years vaccination against PCV2 and M. hyo has both been established as a routine measurement for the majority of fattening pigs in Europe. Both vaccines have to be administered around three weeks of age. Accordingly the administration of a combined PCV2/M.hyo vaccine would offer the following advantages a) Ensurement that pigs are vaccinated at the right time with all relevant vaccines, b) Improvement of animal welfare due to less handling and c) Reduction of costs and resources due to less labour intensive work. Recently, the simultaneous use of Porcilis®PCV with Porclis®M Hyo has been shown to be safe and efficacious under laboratory conditions (1). Moreover, the beneficial aspects of a PCV2 vaccination even in the case of a late occurring PCV2 infection has been reported (2). The objective of this field study was to support these data when testing Porcilis®PCV with or without the addition of Porcilis®M Hyo in the face of a of a late PCV2 infection.

Materials and methods

The study was conducted from June 2009 to March 2010 on a farrow-to-finish farm in Austria, which had experienced severe problems with PCV2 in 2006. The study was conducted as a blinded and randomised field trial. In total, 478 piglets from 5 consecutive batches were assigned to four treatment groups at the age of one week and were followed until slaughter (Table 1).

All animals were weighed at 1, 3, 12 and 25 weeks of age. PCV2 viral load and PCV2 serology was determined at 6, 10, 15, 20 and 25 weeks of age in 15% of the animals. In addition, local reactions after vaccination, mortality and morbidity were recorded.

Table 1: vaccination scheme of the study groups

Group	No of animals	Product:	1st week	3rd week
A	120	Diluvac Forte (Placebo)	X	X
B	120	Porcilis PCV		X
C	119	Porcilis PCV		X*
		Porcilis M Hyo	X	X*
D	119	Porcilis PCV		X**
		Porcilis M Hyo	X	

* Concurrent use: vaccines are given at the same time, but at different sites. ** Simultaneous use: 50ml Porcilis PCV and 50ml Porcilis M. Hyo are mixed in a third 100ml vial and one 4ml injection is given at one site.

Results

Following vaccination, all vaccinated groups showed higher antibody titers than the control animals. The infection period of PCV2 was observed between 20 and 25 weeks of age, as the first animals became PCV2 viremic. During this time, vaccinated animals had lower viral loads than the control group.

In the fattening period, animals vaccinated with Porcilis®PCV gained 14 gram more body weight per day than control animals. An even higher ADWG (22-26 g/day) could be observed when animals received Porcilis®PCV simultaneously or concurrently with Porcilis®M Hyo (Table 2).

No statistical significant differences between the study groups were observed in terms of mortality and morbidity.

Following vaccination, 2 out of 478 animals (one Porcilis®PCV vaccinated and one control animal) showed a transient swelling at the injection site. None of the animals developed any adverse events.

Table 2: Average daily weight gain in g/day and standard deviation *(Difference compared to control group C)*

	Weaning to End (3-25 weeks of age)		Fattening period (12-25 weeks of age)	
Group	ADWG + STD	Diff.[1]	ADWG + STD	Diff.[1]
A	654.6 ± 88.8	-	830.1 ± 132.0	-
B	664.2 ± 76.8	+9.6	844.2 ± 112.1	+14.1
C	665.1 ± 81.0	+10.5	856.8 ± 116.9	+26.7
D	665.3 ± 77.5	+10.7	852.4 ± 121.3	+22.3

[1] Difference: Vaccinated group (B, C, D) minus Control group (A)

Discussion

Even in the face of a late occurring PCV2 infection (between 20th and 25th week of life) vaccination against Porcilis®PCV alone still resulted in an improvement of ADWG. The ADWG could further be increased if Porcilis®PCV was given simultaneously or concurrently with Porcilis®M Hyo. Also the safety of Porcilis®PCV in animals was not negatively influenced by the simultaneous or concurrent use with Porcilis®M Hyo. Accordingly these data indicate that mixing of Porcilis®PCV with Porcilis®M Hyo is safe. In addition, the higher ADWG in the two groups receiving both vaccines suggests that there is no negative interference between Porcilis®PCV and Porcilis®M Hyo but rather an additive effect in terms of efficacy.

Acknowledgements

This work was supported by Intervet/Schering-Plough Animal Health.

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P.068

REPRODUCTIVE FAILURE CAUSED BY PCV2 INFECTION AND FIELD EXPERIENCES WITH PCV2 SOW VACCINATION

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Introduction

Porcine Circovirus type 2 (PCV2) is one of the most relevant pathogens in pig production. It can cause devastating disease in wean-to-finish pigs, and piglet vaccination around weaning has become routine use in many herds. In addition, it has been shown that PCV2 infection can cause reproductive failure (1). This case report describes the field experience with PCV2 sow vaccination on a Mexican farm where PCV2 had been identified as the cause of reproductive failure.

Materials and methods

Late 2007 finishing mortality significantly increased in a PRRS negative 3-site production system of 3,000 sows and PCVAD was diagnosed primarily based on necropsies. Piglet vaccination at weaning (3 weeks of age) was implemented beginning of 2008 and continued since then until today. Average finishing mortality rate was 4.2% pre-PCVAD, increasing to over 12% during the outbreak, while decreasing to 3.6% after piglet vaccination was implemented. In 2009 reproductive failure associated with PCV2 infection was diagnosed. In 28/40 aborted fetuses or mummies submitted for testing necrotic myocarditis was diagnosed. PCV2 was detected by IHC (immunohistochemistry) was detected in 20/28 of affected foetuses.

Following these diagnostic findings sow vaccination was implemented on top of piglet vacciantion. All breeding animals (gilts, sows and boars) in site one were mass vaccinated in week 24 of 2009 with a single dose (1 ml) of Ingelvac CircoFLEX®. Future breeding gilts were vaccinated as piglets and again at about 21 to 22 weeks of age (5 weeks after selection). From week 50 of 2009 onwards, all gilts and sows are vaccinated on a weekly basis 5 weeks pre- farrowing. Since 2008 every other month samples from 10 pigs each at 3, 9, 15 and 20 weeks of age were taken and tested in PCV2 qPCR. Aborted fetuses and mummies were continously submitted to check for pathology findings and PCV2 IHC.

Reproductive parameters were evaluated before (week 1/2008 to week 23/2009) and after (week 24/2009 to week 41/2010) implementation of sow vaccination. Abortion and mummifciation rate were statistically anlalysed using Johnson tranformation method and then ANOVA (SAS 9.2)

Results

No post-vaccination reactions were reported after the mass vaccination procedure.

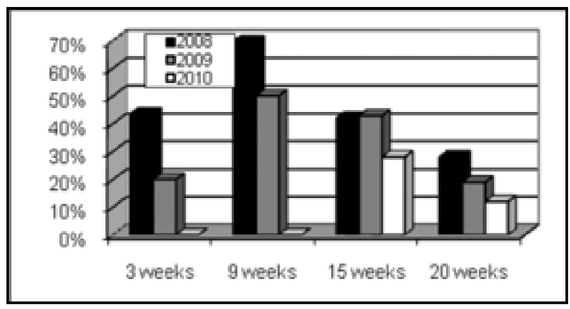
Compared to non-vaccinates sows, vaccinated sows had significantly decreased in percentage abortion and percentage of mummies (Table 1). Since week 42 of 2009 all pigs monitored were negative to qPCR at 3 weeks of age. In 2010 all samples from pigs at 3 and 9 weeks of age tested negative in the qPCR (Figure 1). No evidence of PCV2 lesions in mummies and abortions have been found after sow vaccination was implemented.

Table 1. Abortion and mummification rate.

	Before	After
Abortion rate (%)	3.86a	2.38b
Mummification rate (%)	3.83a	2.54b

ab; $P < 0.05$

Figure 1. PCV2 Prevalence 2008, 2009, 2010



Discussion

PCV2 was identified as the cause of reproductive failure in a 3000 sow production system. Mass vaccination of the sow herd with Ingelvac CircoFLEX proved to be safe. After implementing sow vaccination PCV2 was never found again in abortion material and the prevalence of PCV2 in young pigs clearly decreased, with no PCV2 found anymore in pigs at 3 weeks of age from the end of 2009 onwards. The results indicate that Ingelvac CircoFLEX might be a useful tool to reduce/prevent reproductive failure associated with PCV2 infection.

The economic relevance of PCV2 reproductive failure, especially in comparison with other factors affecting reproductive performance, needs further investigation.

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P.069
COMPARATIVE EFFICACY OF PCV2 VACCINATION PROTOCOLS

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Introduction

Porcine circovirus type 2 (PCV2) is the agent of postweaning multisystemic wasting syndrome (PMWS), the main clinical symptoms are progressive weight loss, dyspnea, enlargement of lymph nodes, diarrhea, pallor, and jaundice (1). Vaccines against PCV2 are now available and used worldwide (2). CIRCOVAC®, an adjuvanted vaccine contains inactivated PCV2 viral particles (3).

In this study we have observed the effects of different vaccination protocols in preventing the impact of the PCV2 infection on growth performances and mortality.

Materials and methods

Three different vaccination protocols were used for three groups of sows and/or their progeny (Table 1). Sows were vaccinated twice, 3-4 weeks apart, before farrowing, whereas piglets were injected at 3 weeks of age with 0.5 ml of the vaccine according to manufacturer´s recommendation (CIRCOVAC®). Control group animals were injected with sterile water as a placebo. The pigs were individually weighted at 1, 3, 10 and 22 weeks of age.

Statistical analysis was done using the software SPSS 15 for Windows. Weight gain and mortality between vaccinated groups were analyzed by analysis of variance (ANOVA) using the General Linear Model (GLM) procedure.

Table 1. Descriptive data of vaccinated groups against PCV2 and control group.

Variable/ vaccinated group	Control	Sows and piglets	Piglets	Sows
Number of sows	18	20	18	20
Number of piglets	164	206	166	200
Sow parity	4.7 ± 1.4	4.6 ± 1.7	4.6 ± 1.5	4.5 ± 1.7
Piglets born alive per litter	10.1 ±1.6	10.7 ±1.8	10.2 ±1.7	10.6 ±1.6

Results

The average of piglets born alive per litter for vaccinated and non-vaccinated sows was 10.6-10.7 and 10.1-10.2, respectively. Table 1 and 2 shows the average daily weight gain and mortality rate of all vaccinated groups.

Table 2. Average daily weight gain (g/day) of piglets during different study intervals.

Vaccinated pigs	Age, weeks ± SD			
	1-3	3-10	10-22	1-22
Control	227.0 ± 26.7	387.3 ± 47.1	860.4 ± 99.9	618.2 ± 55.5
Sows	232.3 ± 27.5	400.4 ± 46.8^a	874.9 ± 108.0	634.0 ± 57.4^a
Piglets	238.0 ± 29.9^a	417.8 ± 54.3^a	883.9 ± 116.6^a	638.8 ± 71.2^a
Sows and piglets	238.6 ± 29.7^a	423.2 ± 57.2^a	916.5 ± 109.7^a	656.2 ± 56.3^a

SD, Standard deviation; $^aP \leq 0.05$ vs Control group.

Table 3. Mortality rates (%) of vaccinated groups against PCV2 and control group.

Vaccinated pigs	Age, weeks ± SD			
	1-3	3-10	10-22	1-22
Sows and piglets	3.74	1.52^a	1.03^a	9.81^a
Piglets	5.66	2.13^a	1.09^a	14.15^a
Sows	8.79	3.33	2.07	21.98
Control	10.87	5.56	2.94	28.26

SD, Standard deviation; $^aP < 0.05$ vs Control group.

Discussion

Sows and/or their progeny vaccinated significantly improve the daily weight gain and reduce the mortality rates. The results obtained in this study indicate that the vaccination of sows and piglets can influence production performance and therefore reduce economic losses from PCV2 infection.

Acknowledgements

This work was supported by the project SEP-México (PROMEP/103.5/10/4959).

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P.070
REDUCTION OF ANTIBIOTICS AFTER IMPLEMENTING PCV2 VACCINATION ON 460 SOW DUTCH PIGFARM

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Introduction

The antibiotic use in the food producing business in the Netherlands is one of the highest in the EU (1,2) and has come under greater governmental attention the last years (3). Vaccination against different diseases can play a vital role in the reduction of the use of antibiotics and at the same time improve the technical performance, resulting in a better economical payoff for the primary producer (4, 5). For PCV2 it is known it can have an immunosuppressive effect (6) and as a result of this secondary (bacterial) infections can have a bigger impact. This study looks at the technical performance and the antibiotic use in the fattening unit following the introduction of a PCV2 vaccine in the nursery.

Materials and methods

Production data of a 460 sow farm was retrospectively reviewed for the period January 2008 until December 2009. Begin 2008 the farm expanded their fattening unit from 1500 to 3500 places (closed herd). At the same time with this expansion, big health problems were seen in the fattening unit, resulting in a high number of runts, mortality, lung problems and big difference in uniformity. These problems did not resolve although a lot of antibiotics were used.

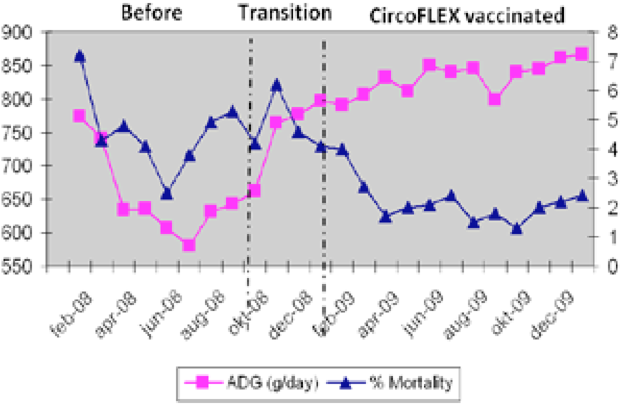
In August 2008 PCV2 was diagnosed as primary agent by multiple necropsies in pigs of 12 to 17 weeks of age (with high viral load) and positive convalescent serology. Immediately Ingelvac CircoFLEX® vaccination (1 ml) was implemented (pigs from 15 weeks backwards to 5 weeks of age were vaccinated at start; from that point pigs from 5 weeks of age for convenience reasons). Continuous flow technical data was used for evaluation, on monthly data basis: 8 months before vaccination- transition period of 4 months – 12 months of vaccinated pigs.

The following parameters were evaluated over time: average daily gain (ADG), mortality and antibiotic use, over the 3 defined periods. The use of antibiotics was measured by Defined Daily Dosages (DDD), the standard method used now in the Netherlands to compare the antibiotic use in time, and between farms (2, 7, 8).

Results

For the 3 consecutive periods there was an increase of the growth (654 *vs*. 747 *vs*. 834 g/d) and a reduction of mortality (4.39 *vs*. 4.9 *vs*. 2.20 %; Figure 1). This was also reflected in clinical healthier pigs, with improved uniformity at time of slaughter. At the same time the production parameters improved, the amount of antibiotics used reduced strongly by -39% (49.87 *vs.* 45.12 *vs.* 30.27 DDD). The vaccinated pigs of the last 8 months had a further improvement in the antibiotic use (18 DDD) compared with the first batch of vaccinated pigs after start.

Figure 1. ADG (g/day) and % mortality on monthly basis before and after implementation of CircoFLEX®



Discussion

The introduction of a PCV2 piglet vaccination resulted in a clear improvement of the technical results as well as a reduction of antibiotic usage in the pigs. The results on this farm are in line with other reports (9,10) showing that by controlling PCV2 with vaccines can help in reducing the use of antibiotics and are a useful tool for the farmer to produce safe food, demanded by the consumers and politics.

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P.071

EFFECT OF FIVE COMMERCIAL PCV2 VACCINES ON WEIGHT AND MORTALITY PARAMETERS

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Introduction

Porcine circovirus type 2 (PCV2) has become one of the most important disease in pigs, is the cause of economic losses throughout the world (1). PCV2 vaccines proved to be an efficient tool to control of porcine circoviral disease (PCVD; 2, 3). The objective of this study was to evaluate the effect of five vaccines market in Mexico on mortality rate and daily weight gain.

Materials and methods

The study was conducted in an age segregated production with strict all/in/all/out regime in the units that experienced severe problems with PCV2. A total of 1950 3-week- old piglets were individually weight (1, 3, 10 and 22 weeks of age) and randomly allocated into 6 groups (Table1). Control group receiving sterile water as a placebo. Statistical analysis was done using the software SPSS 15 for Windows. Weight gain and mortality between groups vaccinated were analyzed using the General Linear Model (GLM) procedure.

Table 1. Experimental design.

Group (vaccine)	Number of pigs	Weeks of age	Dose (ml)
Control	267	3	1
A (inactivated PCV1-2 chimera)	368	3	2
B (inactivated PCV2)	340	3	0.5
C (inactivated PCV2)	288	3 and 6	2
D (PCV2 ORF2 protein)	349	3	1
E (PCV2 ORF2 protein)	338	3 and 6	2

Results

Groups A-D showed a significant increase at the daily weight gain for the study period (3-22 weeks, Table 2). Mortality rates were significantly reduced in all vaccinated groups for the study period (3-22 weeks) than in the control group (Table 3).

Table 2. Average daily weight gain (g/day) during different study intervals.

Group	Weeks of age ± SD			
	1-3	3-10	10-22	3-22
Control	234.3 ± 8.9	376.3 ± 10.2	881.2 ± 9.3	689.4 ± 4.8
A	232.8 ± 7.0	388.3 ± 8.7[a]	876.8 ± 9.9[a]	691.3 ± 6.0[a]
B	234.3 ± 12.2	401.2 ± 14.[a]	881.2 ± 14.7	698.9 ± 7.6[a]
C	235.1 ± 10.2	338.3 ± 7.7[a]	907.5 ± 21.8[a]	691.3 ± 12.7[a]
D	231.2 ± 8.0[a]	419.1 ± 10.[a]	871.8 ± 18.0[a]	699.9 ± 10.2[a]
E	232.9 ± 12.7	340.6 ± 7.3[a]	904.3 ± 17.0[a]	690.2 ± 10.9

SD, Standard deviation; [a]$P \leq 0.05$ vs Control group.

Table 3. Mortality rates (%) of vaccinated groups against PCV2 and control group.

Group	Weeks of age				
	1-3	3-10	10-22	3-22	1-22
Control	10.70	5.62	3.57	9.19	18.73
A	5.64	2.17[a]	1.39[a]	3.56[a]	9.20
B	8.60	3.24[a]	2.43[a]	5.67[a]	14.27
C	14.03	3.82[a]	4.33[a]	8.15[a]	22.18
D	8.16	1.15[a]	1.74[a]	2.89[a]	11.04
E	10.34	1.18[a]	2.40[a]	3.58[a]	13.92

[a]$P \leq 0.05$ vs Control group.

Discussion

PCV2 vaccine is now routinely used in the pig industry. Vaccinated group E with PCV2 ORF2 protein showed the lowest daily weight gain (3-22 weeks). All vaccinated groups of piglets showed significantly reduced mortality rate. Pigs vaccinated with PCV2 ORF2 protein showed more reduced mortality rate for the study period compared with control group.

The results obtained in this study indicate that the vaccination against PCV2 can influence production performance and therefore reduce economic losses.

Acknowledgements

This work was supported by the project SEP-México (PROMEP/103.5/10/4959).

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P.072

IMPACT OF DIFFERENT PCV2 PIGLET VACCINES ON WEIGHT GAIN FOLLOWING VACCINATION

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Introduction

Vaccination of piglets against PCV2 virus has shown effectively to reduce clinical signs of PCVD and improve performance. In Denmark, two vaccines are available, which have been developed for piglet vaccination: Porcilis® PCV and Ingelvac CircoFLEX®. Farmers have to decide which vaccine to use in the field, and the economically most relevant parameter is the impact a vaccine has on the performance. The two piglet PCV2 vaccines have comparable efficacy claims, but for Porcilis PCV, the SPC specifies a risk of impaired growth in the period after vaccination.

Therefore, the present field study was carried out to compare the impact on performance of the two piglet PCV2 vaccines, and this abstract focuses on the performance the first weeks after vaccination.

Materials and methods

The study was carried out in a Danish SPF herd. The study was designed as a double blinded side-by-side (SBS) trial in 4 batches. Each batch included 2 groups vaccinated i.m. against PCV2 at weaning at 4 weeks of age. One group was vaccinated with Ingelvac CircoFLEX® (1 ml), and one group was vaccinated with Porcilis PCV (2 ml). Vaccines were acclimatised to room temperature before injection.

The study included 938 pigs. The pigs were individually earmarked and randomly assigned to the treatment groups at weaning; stratified according to litter, gender and weight. Blood samples were taken from 2 pigs per litter and examined for maternally derived antibodies (MABs) to PCV2 by ELISA (Synbiotics, Bioscreen, Germany).

The pigs were individually weighed at weaning and 2 weeks post weaning. Local reactions on the site of vaccination were recorded for all animals individually as grade A, B, C and D as described by (1) on 1, 2 and 3 weeks post vaccination. Statistical comparison of the groups was done with chi-square test or Mann-Whitney U test with p=0.05 as level of significance.

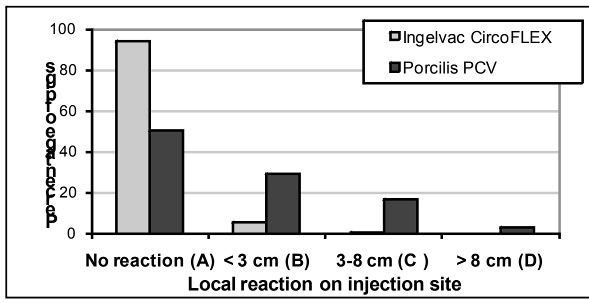
Results

The mean weight gain in pigs vaccinated with Porcilis® PCV was 194 g lower in the first two weeks after weaning than for piglets vaccinated with Ingelvac CircoFLEX® (p=0.0035). Starting weight and gain/day is given in table 1. A low level of PCV2 antibodies (< 5 log2) was found in 26% of the blood samples, and 42% had a high level (> 10 log2), evenly distributed between groups.

Table 1. Weight and weight gain post vaccination.

Vaccine	# pigs	Weaning weight (kg)	Weight gain g/day 2 weeks post wean
Porcilis PCV	460	6.026	205.7
CircoFLEX	478	6.034	219.8
Diff PP-CF	-	0.008	-14.1*

* = Result in the 2 groups significantly different (p<0.05)



In the group vaccinated with Porcilis® PCV, 58% had local reactions on the injection site, this was significantly more than in the group vaccinated with Ingelvac CircoFLEX®, where only 12% had local reactions (p<0.0001). The highest frequency of local reactions was seen 2 weeks after vaccination (Figure 1).

Figure 1. Local reaction on injection site 2 weeks post vaccination (926 pigs).

A: No reaction; B: < 3 cm diameter; C: 3-8 cm diameter; D: >8 cm diameter.

Discussion

This blinded SBS study showed that Porcilis® PCV had a negative impact on the weight gain following vaccination. Weight gain was significantly higher in the Ingelvac CircoFLEX® group, indicating that weight gain after weaning was not impaired by this vaccine; confirming previous publications (2). The reduced performance in pigs vaccinated with Porcilis®PCV might be related to the large number of local reactions. In the Ingelvac CircoFLEX® group, very few pigs showed local reactions, all limited in size. Weaning is a critical time for pigs, and unnecessary stress should be avoided. The results shown here indicate that the choice of PCV2 vaccine has an impact on performance after weaning, which might lead to poor performance through to finishing.

Acknowledgements

Thanks to DVM Elizabeth Mortensen and Vet. Stud. Christina Jensen for carrying out the practical work.

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P.073

A FOLLOW UP IN TIME OF THE EFFECT OF A ONE DOSE PORCINE CIRCOVIRUS 2 (PCV2) VACCINE ON PCV2 INFECTION MEASURED BY QUANTIFICATION OF THE VIRAL LOAD AND PRRSV INFECTION MEASURED BY DETECTION OF ANTIBODIES.

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Introduction

Porcine circovirus type 2 (PCV2) is the causative agent of several diseases that are grouped as porcine circovirus(-associated) disease (PCV(A)D) (1). However, the risk for clinical PCV(A)D seems to be much higher if the pig is coinfected with PRRSV (2). Since the introduction of vaccines against PCV2 many reports from the field suggest that PCV2 vaccines decreases significantly mortality rates and improve growth performances (3,4). The present field study aims to investigate if PCV2 vaccination has an influence on PCV2/PRRSV coinfection

Materials and methods

On a farrow to finish farm 56 three-week old piglets were divided into two groups: 27 piglets were vaccinated with Ingelvac® CircoFLEX™ and 29 piglets were used as control. In each group 10 ad random selected piglets were labeled and serum samples were taken every four weeks starting from the day of vaccination for analysis. For quantification of the PCV2 viral load serum samples were analyzed by an ORF2-based real-time qPCR developed in our laboratory and modified from previously described techniques. PRRS virus circulation was followed up by measuring the antibodies response with an ELISA (IDEXX HerdChek PRRS 2XR ELISA).

Results

Mean viral load of the 10 vaccinated piglets was significant lower at week 4, 8 and 16 post PCV2 vaccination (P< 0.05) (Figure 1). Except for one vaccinated piglet at week 12, the vaccinated piglets didn't showed detectable viral load (detection limit = 3 log10 genome copies/ml serum). Four weeks post PCV2 vaccination all the piglets from the vaccinated and the control group had an S/P ratio >1.0 indicating that PRRSV was circulating (figure 2). The S/P ratios diminished on week 16 but on week 20 they were significant higher. Between the PCV2 vaccinated and the control group there was no significant difference in s/p ratio during the whole period.

Discussion

Previously it was shown that PCV2 vaccines decreases significantly mortality rates and growth performances (3,4). In this field study we demonstrated a significant difference in viral load between the PCV2 vaccinated group and the control group. A significant difference between the PCV2 vaccinated group and the control group could not be observed for PRRSV circulation as measured by s/rp ratio.

Surprisingly the S/P ratios raised again on week 20, suggesting that the pigs were infected for a second time. Further investigations are now done to characterize the viruses circulating at the beginning and at the end of this study.

Figure 1. Follow up of the mean viral load (log10 genome copies/ml) of a PCV2 vaccinated group and a control group in time.

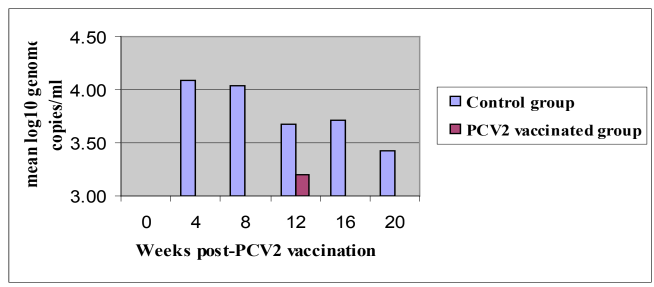
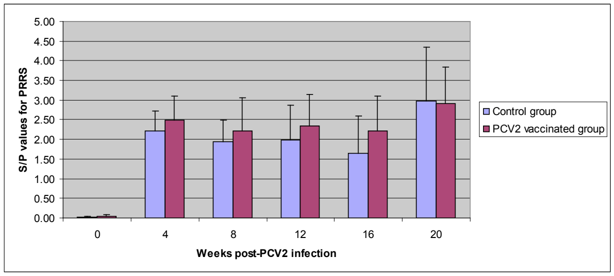


Figure 2. Follow up of S/P ratio's for PRRSV of a PCV2 vaccinated group and a control group in time.



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P.074

FIELD EXPERIENCE WITH THE MIXTURE OF INGELVAC CIRCOFLEX® AND INGELVAC MYCOFLEX® ON A 1250-HEAD SOW FARM IN SPAIN

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Introduction

With the growing number of vaccinations applied to piglets, vaccine combinations, that allow to reduce the number of injections, become more and more important. Recently, Ingelvac CircoFLEX has been granted a label extension that allows its mixing with Ingelvac MycoFLEX (1). With a single injection pigs can be protected against two of the most important pathogens in Spain: Porcine Circovirus type 2 (PCV2) and Mycoplasma hyopneumoniae (M. hyo). The objective of this field observation was to evaluate if Ingelvac CircoFLEX® mixed with Ingelvac MycoFLEX® before use (here referred to as FLEXcombo®) is as effective as a programme using Ingelvac CircoFLEX and a 2-dose M. hyo vaccine.

Materials and methods

The study farm is a two-site, 1250-head sow farm. The farm is positive for PCV2, M hyo as well as PRRS, APP and P. multocida. Pigs were frequently suffering from respiratory clinical signs due to secondary bacterial infections at the beginning and at the end of the grow-finish period. Wasting, with sudden impairment in growth, was observed when pigs were weighing about 80 kg. Pigs were vaccinated at 3 weeks of age with Ingelvac CircoFLEX and at 3 weeks and 6 weeks of age with 2-ds M. hyo vaccine (n=11.600). The first shot of the M. hyo vaccine was given concurrently with Ingelvac CircoFLEX. Later that year, after the label claim to mix Ingelvac CircoFLEX with Ingelvac MycoFLEX was granted, the programme was switched to FLEXcombo for convenience reasons. From mid 2010 pigs were vaccinated with a single dose of FLEXcombo (2ml) at 3 weeks of age.

Parameters evaluated included the weight at entry into the grow-finish farm, the weight at slaughter, the number of days pigs spent in the farm, the average daily gain (ADG) and feed conversion ratio (FCR). Animals remaining on the farm, when the barn is emptied, are classified as lightweight pigs, while pigs weighing less than 85 kg at the end of finishing are classified as culls. Variables were analysed using the general linear model procedure (SAS, Cary, NC), including entry weight as a covariate. Mortality, culls and lightweights were analysed using a Chi-square test.

Results

Performance results in grow-finish comparing the previously used vaccination schedule with the mixture of Ingelvac CircoFLEX and Ingelvac MycoFLEX are shown in table 1. No adverse reactions were observed after adminstration of FLEXcombo®.

Table 1. Performance parameters in grow-finish.

Parameter	CircoFLEX + 2-ds M hyo vx	FLEX-combo
Number of pigs	11.600	4.200
Number batches	9	4
Entry weight (kg)*	23[a]	26[b]
Slaughter weight (kg)*	102	103
Days in grow-finish*	128	120
ADG (g/day)*	741	732
FCR*	2,27	2,33
Mortality (%)	4,17	3,53
Culls (%)	8,35[a]	4,44[b]
Lightweights (%)	1,81[a]	1,36[b]
Medication costs (€/pig)*	1,36	1,21

a,b: values with a different superscript, differ significantly ($p < 0.05$) *batch as experimental unit

Discussion

Ingelvac CircoFLEX mixed with Ingelvac MycoFLEX and applied as a single 2ml injection produced comparable performance results as a vaccination programme using Ingelvac CircoFLEX and a 2-ds M. hyo vaccine. The results indicate that the mixture was at least as effective to control PCV2 and M. hyo as the separate application of Ingelvac CircoFLEX and the 2-ds vaccine. The number of culls (%) and lightweights (%) were significantly reduced and the days in grow-finish and mortality were clearly reduced (tendency, p=0.06 and p= 0.08) in the pigs vaccinated with the mixture. This could be an effect of less stress for pigs around weaning, with only one injection in the FLEXcombo group compared to 3 injections with the previous programme. Or it might indicate that pigs were better protected against the common pathogens of the farm.

The FLEXcombo® concept is a helpful tool to reduce stress for pigs and labour for the producer while retaining at least the same level of efficacy compared to a programme with a total of three injections applying two vaccines separately.

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P.075

EXPERIENCE WITH PCV2 AND MYCOPLASMA HYOPNEUMONIAE VACCINE MIXTURES IN A FARM AFFECTED BY E. COLI AROUND WEANING – A CASE REPORT

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Introduction

PCV2 and Mycoplasma hyopneumoniae (M hyo) vaccines in the field are being mixed with or without authorization. In mid 2010 in Europe, Ingelvac CircoFLEX® has been granted a label extension that allows its mixing with Ingelvac MycoFLEX® before use. This concept is referred to in this abstract as FLEXcombo®. While FLEXcombo® has been shown to be very easy on the pigs, other non-authorized vaccine mixtures are more reactive (1), which might have a negative impact on the pigs performance.

The objective of this study was to compare the performance results using FLEXcombo® with the PCV2/M hyo vaccination program previously used on the farm.

Materials and methods

The study farm was a two-site, 1000 head sow farm, being positive for PRRS, PCV2 and M hyo. At 3 weeks of age animals are weaned and moved to the nursery site. During the study, 519 piglets from one batch were divided in two groups and were vaccinated at 3 weeks of age (day 0): in one group, 258 piglets were vaccinated with a single injection (2 ml) of FLEXcombo®. In the other group, 261 piglets were vaccinated with a single injection (4 ml) of a non authorized mixture of a mineral oil based PCV2 vaccine and a *M. hyopneumoniae* vaccine of the same manufacturer.

Animals were weighted individually at day 0 and 14 of study and body temperature before and 8 hours after vaccination, as well as common production parameters were registered. Weight gain variables were analysed using the general linear model procedure (SAS, Cary, NC) including weight on day 0 as a covariate. Mortality and number of pigs moved to the hospital pen were analyzed by Chi-square test.

Results

Weight gain and temperature results are shown in table 1 and 2. No adverse reactions were observed after administration of FLEXcombo®. Nine animals vaccinated with the non-authorized mixture suffered from systemic reactions after vaccination (6 vomiting, 2 had convulsions and 1 with both). Three days after vaccination, an outbreak of diarrhoea (clinically compatible with E. coli) occurred in the farm, 75-80% of the animals vaccinated with the non authorized mixture needed to be treated. In the FLEXcombo group, 5-10% of the animals needed individual treatment for diarrhoea.

Table 1. Weight gain and ADG 14 days post-vaccination.

Treatment	Weight d0	Weight d14	ADG 0-14	Weight gain 0-14 (kg)
Non-author. mixture	5.18^a	6.83^a	0.118^a	1.65^a
FLEXcombo	5.62^b	7.50^b	0.134^b	1.88^b

a,b: values with a different superscript, differ significantly ($p < 0.05$)

Table 2. Body temperature at day 0.

Treatment	Before vx (t0)	8h after vx (t8)	Increase (t8-t0)
Non-author. mixture	39.76	41.22^a	1.46
FLEXcombo	39.72	39.70^b	-0.02

a,b: values with a different superscript, differ significantly ($p < 0.0001$)

At the end of nursery period, animals of FLEXcombo group had less mortality (1.94% vs 3.83%) and significantly less animals moved to hospital pens after the E. coli outbreak (11.63% vs in 19.54%).

Discussion

During the study the nursery unit was affected by an *E. coli* outbreak. Animals vaccinated with the non-authorized mixture suffered significantly more from the disease as the ones vaccinated with FLEXcombo®. This was reflected in more animals treated and moved to the hospital pens. Mortality was higher in the group vaccinated with the 4 ml non-authorized mixture and weight gain in the 2 weeks post vaccination was reduced significantly.

The differences observed between the treatment groups after the E. coli outbreak are probably related to the adverse events seen immediately after vaccination. In the group injected with the non-authorized mixture 9 animals showed systemic reactions and temperature increased by about 1.5°C.

No adverse reactions were observed in animals vaccinated with FLEXcombo® and body temperature remained. The results indicate that the non-authorized vaccine mixture had a negative impact on the pigs, having a devastating effect when pigs were affected by E. coli. FLEXcombo proved again to be very safe and less stressful for pigs that other vaccine mixtures. Beside efficacy, safety is a relevant parameter for a vaccine to achieve optimal results.

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P.076

EFFICACY OF THE MIXTURE OF INGELVAC CIRCOFLEX® AND INGELVAC MYCOFLEX® ON A 1200-HEAD SOW FARM GILT PRODUCER

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Introduction

Efficacy of the recently authorized mixture of PCV2 and Mycoplasma vaccines in Europe, FLEXcombo® in fattening pigs has been demonstrated in the field (1,2). The effect of the vaccines against both pathogens in gilts have been proved and experienced in field with success.

The objective of this field study was to evaluate the efficacy of Ingelvac CircoFLEX® mixed with Ingelvac MycoFLEX® before use (FLEXcombo®) against Swine Enzootic Pneumonia and PCVD on productive performance of a gilt production system in Spain.

Materials and methods

The study farm was a three sites 1200 head sow farm that is a Gilt producer (Landrace x Duroc x Large White). Vaccinated pigs with one dose of FLEXcombo® 2ml at weaning (3 weeks of age) were transferred to eight fattening farms. After the fattening period, gilts were sold and males were slaughtered Vaccines against Enzootic Pneumonia and PCVD had not been used in the piglets (sows were vaccinated against PCV2 since late 2007) in the previous batches although mild signs of PCVD and respiratory disease were commonly observed. For each vaccinated fattening batch (8 vaccinated batches entered in fattening from April to August), data from two batches before the implementation of vaccination was compared: pigs of nine non-vaccinated batches were transferred between March and September of 2009 and pigs of seven non-vaccinated batches between October of 2009 and February of 2010 (more than 25.000 animals in total in the study). The parameters analyzed were mortality rate, average daily gain, feed conversion ratio and cost of medication per pig. The statistical procedure used was the variance analysis. Each batch was the experimental unit and the effects included in the statistical model were: vaccination (yes or no) and the season when pigs were transferred to fattening (March – September or October – February).

Results

Performance results in fattening comparing the non vaccinated animals and the vaccinated are shown in table 1. No adverse reactions were observed after adminstration of FLEXcombo®.

Pigs were transferred to fattening farm with similar bodyweight before and after the implementation of vaccination (24.6 kg ± 0.5 vs 23.3 kg ± 0.8, respectively; p>0.05), remained in fattening 110.0 days ± 1.4 vs 107.4 days ± 2.3, respectively (p>0.05) and went out with similar bodyweight (108.5 kg ± 1.6 vs 108.1 kg ± 2.7, respectively; p>0.05).

Mortality rate decreased 2.4% (p<0.05) after the implementation of vaccination and there were no statistically significant changes in the analyzed parameters related to productive performance.

Table 1. Productive parameters of the non-vaccinated (16) and vaccinated (8) batches. Least square mean ± standard error.

	Non-vaccinated	Vaccinated[1]
Mortality rate (%)	5.4^a±0.5	3.0^b±0.9
Average daily gain (g)	763±14	789±23
Feed conversion ratio (kg/kg)	2.80±0.04	2.73±0.06
Cost of medication per pig (€)	1.82±0.20	1.89±0.34

a,b: values with a different superscript, differ significantly
*batch as experimental unit

Discussion

The results indicate that the mixture of Ingelvac CircoFLEX® mixed with Ingelvac MycoFLEX® before use (FLEXcombo®) is efficacious in the performance of fattening of the gilts. No clinical signs of PCVD and respiratory diseases were observed during fattening since the vaccination was started in the system. Mortality was significantly reduced by 2.4% and production parameters were improved (+26g/day of ADG and 70g less of FCR) in vaccinated animals with FLEXcombo®.

Although the season effect has been included in the analysis, this has been done with the batch as the experimental unit. Further data in this system is needed to evaluate a whole year results, including more batches of vaccinated animals (transferred in winter time) that could demonstrate that this important numeric differences in ADG and FCR are statistically significant.

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P.077

ADDITIONAL BENEFITS PROVIDED BY VACCINATION AGAINST PCV2 AND MYCOPLASMA HYOPNEUMONIAE WITH FLEXCOMBO® IN A COMMERCIAL IBERIAN HERD

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Introduction

Vaccines against *Mycoplasma hyopneumoniae* (M. hyo) and PCV2 are commonly used in Spanish pig production. A speciality of Spanish pork production is Iberian ham. For that, pigs are slaughtered at about 150kg live weight with a minimum age of 10 month. A concern for Iberian pig producers is the duration of protection provided by commercial vaccines. Recently Ingelvac CircoFLEX® was licensed to be mixed with Ingelvac MycoFLEX® before use. The efficacy and safety of this mixture had been demonstrated in the field and documented in several laboratory and field studies (1, 2, 3, 4, 5).
The objective of this study was to evaluate if vaccination with Ingelvac CircoFLEX® mixed with Ingelvac MycoFLEX® (FLEXcombo®) against PCV2 and M. hyo protects pigs through to slaughter under the conditions of Iberian pig production and provides additional benefits compared to M. hyo vaccination alone.

Materials and methods

This study was conducted in a 2400-sows, commercial Iberian herd in Salamanca, Spain. It was negative for PRRS, positive for M. hyo and positive for PCV2 in a subclinical form. Pigs are weaned weekly at 3 weeks of age and are slaughtered with a minimum age of 10 months (43 weeks of age). In this study a total of 2.020 piglets were randomly divided into two groups at weaning, individually ear-tagged and vaccinated with either 2 ml of Ingelvac M. hyo® (group 1, n=1,020) or with 2 ml of FLEXcombo® (group 2, n=1,000). Piglets remained in the nursery farm until they reached approximately 26 kg of live weight. Then they were moved to two identical fattening barns with liquid feed. The barns were on the same site, 40 metres apart. Both groups were fed with the same feed curve, each pen of pigs receiving the same amount of feed. Animals of both groups were slaughtered at the same age. Pigs were individually weighed at the end of nursery and at slaughter. Chi-square test was used to analyse mortality, student's t-test to compare total weight gain (TWG), average daily gain (ADG), and feed conversion ratio (FCR) using SAS as the statistical programme. Furthermore, an economical analysis was made based on the differences in mortality and weight gain, taking into account the higher costs for the mixture, compared to the M. hyo vaccine alone.

Results

No adverse reactions were observed after vaccination with FLEXcombo®. Performance results of the two groups are summarized in Table 1.

All the analyzed parameters (mortality, TWG, ADG and FCR) were significantly improved in the FLEXcombo® group ($p<0.05$). The mortality rate was reduced by 76%. Total weight gained was 3.5 kg more per pig in group 2. As the average daily feed intake was the same in both groups, FCR was improved in FLEXcombo® vaccinated pigs (4.08 in group 2 vs 4.19 in group 1). The ADG was better in group 2 (625g) compared to group 1 (609g). The net advantage for vaccinating with FLEXcombo® was calculated to be 5.04 € per pig.

Table 1: Production parameters for Ingelvac Myo® vs FlexCombo® after vaccination.

	Ingelvac M. hyo® (Group 1)	FLEXcombo® (Group 2)
Pigs	1,020	1,000
Weight in	26.80 kg	26.50 kg
Weight out	153.40 kg	156.60 kg
Mortality	4.80 %	1.40 %
TWG	126.6 kg	130.1 kg
ADG	609 g	625 g
FCR	4.19	4.08

Discussion

In the fattening unit animals vaccinated with FLEXcombo® around weaning performed better than animals vaccinated with Ingelvac M. hyo® alone. This study proved that FLEXcombo® vaccination applied to Iberian pigs can improve the good productive parameters obtained with Ingelvac M. hyo® vaccination in this specialized production system. FLEXcombo® protects against enzootic pneumonia and PCV2 pathology in pigs slaughtered with more than 43 weeks of age. The additional benefit provided by vaccinating with FLEXcombo® was calculated to be as high as 5.04 € per pig.

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P.078
COMPARATIVE REACTIVITY OF COMMERCIALLY AVAILABLE PCV2 VACCINES

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Introduction

Previous studies have demonstrated significant differences in reactivity (side effects described as pig lethargy or "buzzing") of *Mycoplasma hyopneumoniae* (*M. hyo*) vaccines given alone or as mixtures with PCV2 vaccines as measured by pigs' willingness to approach an observer 6 hours post-injection (1,2). The objective of this study was to determine if significant differences in PCV2 vaccine reactivity could be detected using these same techniques.

Materials and methods

Pigs weaned at 16-24 days of age were obtained from a PRRS negative, *M. hyo* positive sow farm. Pigs were randomly assigned by pen to one of three treatment groups balancing for age at weaning and body weight at placement.

A total of 39 pens were populated with an average of 30 pigs per pen for a total of 13 pens per treatment group with pen as the experimental unit. Treatment pens were separated by empty pens. Treatment group 1 (CTRL) was given 1ml of sterile saline by intramuscular injection (IM) at three and six weeks of age. Treatment group 2 (CF) was given the approved 1ml dose of Ingelvac CircoFLEX® (Boehringer Ingelheim Vetmedica Inc, St Joseph, Missouri, USA) IM at three weeks of age. Treatment group 3 (CV) was given the approved 2ml dose of Circumvent® PCV (Intervet/Schering-Plough Animal Health, Kenilworth, New Jersey, USA) IM at three and six weeks of age. Pigs were evaluated to determine willingness to approach (WTA) behavior before and after vaccination using the Swine Welfare Assurance Program swine behavior protocol (3).

After entering and kneeling down in a pen, the blinded observer counted all pigs that approached him/her during a 15 second period. The pigs approaching the observer during this period of time were designated as "willing to approach". WTA was assessed the day before each vaccination (baseline) and again 24 hours later (post-vacc). Vaccination occurred 6 hours prior to the second observation. Treatment group 2, which did not receive a second injection, were measured on the same schedule as groups 1 and 3. The difference in the baseline and post-injection values is reported here as percent difference in WTA (Δ). Results were considered significant when P values were ≤0.05.

Results

Baseline percent WTA did not differ significantly among the 3 groups at either baseline measurement. Groups one (saline, CTRL) and two (Ingelvac CircoFLEX, CF) were not statistically different from each other in percentage change in WTA in either post-injection observation. Treatment group three (Circumvent PCV, CV) was statistically different from both of the other treatment groups in percentage WTA in both post-injection observations.

Table 1. Changes in pigs' willingness to approach due to vaccination with PCV2 vaccines or saline.

	CTRL	CF	CV
No. pens	13	13	13
Avg no. pigs/pen	30	30	30
1st baseline WTA %	66.38[a]	66.54[a]	65.00[a]
1st post-vacc WTA %	52.67[a]	58.75[a]	34.66[b]
1st Δ WTA %	**-13.38[a]**	**-7.22[a]**	**-31.10[b]**
2nd baseline WTA %	56.08[a]	60.23[a]	59.46[a]
2nd post-vacc WTA %	55.98[a]	60.35[a]	46.82[b]
2nd Δ WTA %	**-2.40[a]**	**+1.77[a]**	**-11.91[b]**

WTA: Willingness to approach
[ab]Means differ (Tukey's HSD P≤0.05).

Analyzed using one-way ANCOVA.
Model included vaccine treatment and baseline pre-injection behavior as a covariate.

Discussion

Results of the trial indicate that vaccination with Circumvent PCV caused a decrease in pig activity 6 hours after each vaccination as measured by pigs' willingness to approach an observer. Pigs vaccinated with Ingelvac CircoFLEX showed no statistical difference in willingness to approach from those vaccinated with saline. Outcomes using this method of assessing comparative PCV2 vaccine reactivity were consistent with previous results assessing *M. hyo* vaccine reactivity given alone or as mixtures with PCV2 vaccine (1,2).

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P.079

EFFECT OF ONE OR TWO DOSE VACCINATION REGIMENS ON PCV2 VIREMIA AND ADG IN 5 DIFFERENT US STUDIES

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Introduction

Recently there have been reports suggesting that residual PCV2 viremia in vaccinated pigs could have an impact on performance parameters, and should thus be considered when evaluating the commercial vaccines on the market. Many studies have shown that, in *non-vaccinated* animals, there was often a correlation between the quantity of virus found in tissues and serum, and the severity of clinical signs and lesions.[1,2] Some of these publications have suggested that when a threshold level of virus in tissues is exceeded, the likelihood of developing PCVAD was increased.[1,2] However in *vaccinated* pigs, such a relationship between residual viremia and disease, or between viremia and reduced performance, has notably been absent.[3,4]

There is also some debate on the comparative capability of one vs two dose vaccines and vaccination protocols for reducing residual PCV2 viremia. The objective of this 5 study summary is to evaluate the comparative reduction in viremia and the presence or absence of correlation with ADG.

Materials and methods

This paper summarizes information from 5 different US studies where pigs vaccinated with a two-dose PCV2 vaccine were compared to pigs vaccinated with a single dose of Ingelvac CircoFLEX®. Outcomes of interest are percentage of viremic pigs, average PCR log titer and ADG. Individual pig was the experimental unit in all studies. The number of pigs in each group for each study varies from 120 to 900. Pigs were individually weighed at 3, 10 and 22 weeks of age, and blood sampled at 3, 6, 10, 14, 18 and 22 pigs of age for PCR testing. Each study was statistically analyzed using methods appropriate for each parameter.

Results

The results obtained with two full doses of the two-dose vaccine were not significantly different from those obtained with one dose of Ingelvac CircoFLEX in terms of percentage of viremic pigs, average titer of virus in serum and ADG in any of the 5 studies. Even for studies 3 and 5, where there was an apparent nominal difference in the percentage of viremic pigs and in viral load, there were no differences in ADG.

Table 1: Comparative viremia and weight gain results obtained with Ingelvac CircoFLEX and a two-dose PCV2 vaccine in 5 different US studies.

Study	Two-dose vaccine			Ingelvac CircoFLEX		
	% viremic*	Avg log titer	ADG, lbs	% viremic*	Avg log titer	ADG, lbs
1	**54**	4.99	1.63	**50**	4.87	1.61
2	**0**	4	1.55	**10**	4.11	1.57
3	**7**	4.12	1.62	**33**	4.63	1.63
4	**0**	4	1.34	**8**	4.11	1.36
5	**25**	4.31	1.43	**4**	4.07	1.44
Avg	**17.2**	**4.28**	**1.51**	**21**	**4.36**	**1.52**

*% pigs > 4 logs at peak of viremia.
No statistically significant differences were detected for these parameters within any of the 5 studies.

Discussion

Viremia and ADG did not differ significantly between groups within any of the 5 studies. Even if there had been a difference in viremia outcomes in favor of the same product for all the trials, these criteria are not biologically or economically relevant in the absence of any association with ADG. The choice of PCV2 vaccine should be based on cost-effectiveness, safety, convenience and the potential impact on animal welfare.

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P.080

INFLUENCE OF VACCINATION TIMING ON THE EFFICACY OF A COMBINED MYCOPLASMA HYOPNEUMONIAE AND PCV2 VACCINATION

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Introduction

In 2010 a claim was granted by the EMEA that allows Ingelvac CircoFLEX® to be mixed with Ingelvac MycoFLEX®. Piglets can be protected with a single injection against two of the most common pathogens in France: *Mycoplasma hyopneumoniae* (M hyo) and Porcine Circovirus type 2 (PCV2). For Ingelvac CircoFLEX® it has been shown previously that vaccination at 6 weeks of age is not more effective than vaccination at 3 weeks, demonstrating that efficacy is independent of maternal immunity (1). The objective of this study was to investigate the possible impact of maternal immunity on the PCV2/M hyo vaccine mixture by comparing different vaccination time points. This investigation was primarily focussed on the M hyo component.

Materials and methods

The study was conducted on a farrow-to-finish, PRRS-negative 2.300 sow farm. Two batches of piglets were included in the study at weaning (3 weeks of age). Piglets were randomly divided among 3 treatment groups and vaccinated with a single dose of 2 ml of the mixture of Ingelvac CircoFLEX® and Ingelvac MycoFLEX® (here referred to as FLEX- combo®) at either 3 weeks (n=700, V3), 5 weeks (n=699, V5) or 7 weeks of age (n=698, V7). Twenty unvaccinated sentinel piglets were used to demonstrate presence of PCV2 and M hyo infection during the trial. Average daily gain (ADG) was calculated based on the individual weights at weaning and carcass weights. Mortality was recorded from weaning to slaughter.

Lung lesions scores were evaluated at the slaughterhouse by a person blinded to the treatment using the Madec and Kobisch (2) score (out of 28). To analyse maternal immunity against M hyo 20% of the piglets of each treatment group were bled on the day of vaccination and tested for antibodies with the IDEXX Elisa. Furthermore, 10 samples per batch and treatment group (incl. sentinels) were taken at the end of nursery, mid and end of finishing and tested for M hyo antibodies. Serum samples of sentinels were tested for presence of PCV2 by PCR. Statistical analysis was carried out with SAS v8.2. ADG and carcass weights were compared by one-way ANOVA, and lung lesion score by Cochran-Mantel-Hanszel, adjusted for batch and sex. Mortality was analysed with Fisher's exact test.

Results

During the trial M hyo infection was present as confirmed by positive results in the M hyo antibody Elisa at the end of finishing. PCV2 antigen was detected in serum samples of sentinels mid and end finishing. The prevalence of M hyo antibodies at vaccination is shown in Figure 1. Performance and lung lesion results are summarized in Table 1, with no significant differences (p>0.05) being observed between the three treatment groups.

Figure 1. Prevalence of M hyo antibodies (% positive) and average S/P ratio of M hyo positive animals at vaccination.

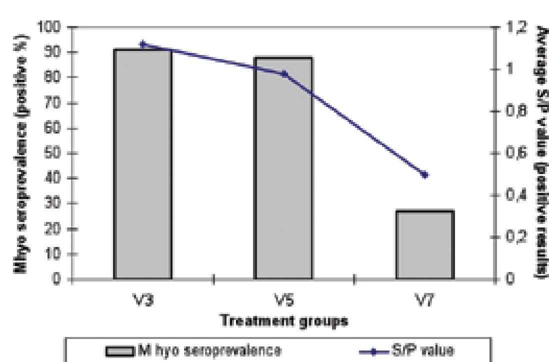

Table 1. Performance results of treatment groups.

	V3	V5	V7
Weight at inclusion (kg)	5,73	5,72	5,76
Mortality (%)	2,29	3,29	2,14
# pigs at slaughter	589	514	561
Carcass weight (kg)	88,53	88,44	87,85
ADG (g/day)	729	726	725
Lung lesion score (/28)	3,0	2,7	2,8

Discussion

No difference in performance, nor in lung lesions was observed between the 3 treatment groups in this PCV2 and M hyo infected herd. Vaccination with FLEXcombo® at 3 weeks of age, in presence of high prevalence of M hyo antibodies, was as effective as vaccination at 5 or 7 weeks, with lower prevalence of M hyo antibodies. It can be concluded that FLEXcombo® is effective in presence of high level of M hyo immunity. It should be noted that both PCV2 and M hyo infection occurred on this farm in finishing only. In case of earlier infection vaccination at 5 or 7 weeks of age might be too late.

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P.081

IMPACT OF DIFFERENT PCV2 AND MYCOPLASMA HYOPNEUMONIAE VACCINE MIXTURES ON PERFORMANCE IN THE PERIOD FOLLOWING VACCINATION

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Introduction

Nowadays PCV2 vaccination is widely used and is being implemented in every single piglet vaccination protocol, alone or combined with a Mycoplasma hyopneumoniae (M. hyo) vaccine. In some farms, adverse events (local and systemic) have been observed during vaccination at weaning with some PCV2 vaccines, more frequently with the ones containing oil as adjuvant (1,2). Impairment of growth is mentioned in the product information of a mineral-oil based PCV2 vaccine. In mid 2010 in Europe, Ingelvac CircoFLEX® has been granted a label extension that allows mixing with Ingelvac MycoFLEX®. The objective of this study was to compare the performance results after applying Ingelvac CircoFLEX® mixed with Ingelvac MycoFLEX® (referred to in this abstract as FLEXcombo®) with the previously used PCV2/M hyo vaccination program in field conditions.

Materials and methods

The study farm is a one site, 350 head sow farm. The farm is positive for PRRS, PCV2 and M hyo as well as APP. The farm works in 4-weekly sow batches and piglets are weaned at 3 weeks of age. Almost 25 piglets per sow per year have been weaned during 2010 in this farm. At 3 weeks of age animals are moved to a nursery (day 0), where the study was performed.

During the study, one batch of piglets was divided in two groups: in one group, piglets were vaccinated with FLEXcombo®, 2ml in one injection. In the other group, piglets were vaccinated with a non authorized mixture of a mineral oil based PCV2 vaccine and a M. hyo vaccine of the same manufacturer, 4ml in one injection (protocol previously used on that farm).

Animals were weighted individually at day 0 and day 14 post-vaccination. Body temperature was measured before vaccination and 8 hours after vaccination. Feed intake was controlled per pen during 2d post-vaccination. Pigs were observed for systemic reactions immediately after vaccination. Variables were analysed using the general linear model procedure (SAS, Cary, NC) including weight on day 0 as a covariate.

Results

Performance results after vaccination comparing the non authorized mixture with FLEXcombo® are shown in table 1. No adverse reactions were observed after administration of FLEXcombo®. In the group vaccinated with the non-authorized mixture 13 animals suffered from systemic reactions after vaccination (5 vomiting and 8 with deep depression).

Table 1. Performance parameters after vaccination.

Parameter	Non authorized mixture	FLEX-combo
Number of pigs	197	207
Body temperature before vaccination	39.68	39.87
Body temperature 8h post-vaccination	41.15^{a}	40.10^{b}
Weight d 0 (kg)	6.4^{a}	6.0^{b}
Weight d 14 (kg)	7.8	7.5
Weight gain 0-14 (kg)	1.43	1.52
ADG 0-14 (g/day)	102	109
Feed intake (g/pig) (48h)	128	150

a,b: values with a different superscript, differ significantly (p< 0.05)

Discussion

Animals vaccinated with the non-authorized mixture suffered from post-vaccination adverse reactions: systemic reactions were observed, body temperature was significantly higher (+1.42ºC) at 8h post vaccination and feed intake was reduced at least during 2 days post-vaccination.

Taking into account the difference on the weight on day 0, lower in FLEXcombo group, weight gain and ADG 14 days post-vaccination was higher (tendency, p<0.08) showing an effect of the post-vaccination reactions.

No adverse reactions and no relevant changes in body temperature were observed in animals vaccinated with FLEXcombo®, 2ml in one injection site. The results confirm that the mixture of Ingelvac CircoFLEX® and Ingelvac MycoFLEX® is very safe, as shown previously (4). This field study indicates that it is less reactive than the non authorized mixture used on the farm previously. Further field trials are needed to evaluate the possible long term consequences of the adverse events seen with the non-authorized mixture.

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P.082
SAFETY, RISKS AND POTENTIAL BENEFITS OF USE OF LIVE ORAL CHIMERIC PCV1-2 VACCINE

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Introduction

Commercial PCV2 vaccines for use in growing pigs and breeding age animals were introduced in North America in 2006 and to date at least 4 products are available. All commercial products to date are inactivated vaccines based on the PCV2a subtype even though the currently dominant strains circulating in North America are PCV2b. Studies have shown that PCV2a and PCV2b are cross-protective (1, 2). It has recently been demonstrated that a novel chimeric porcine circovirus (PCV1-2b) is attenuated *in vivo* and induces protective immunity against PCV2 in high health pigs singularly-challenged with PCV2 (3).

The objective of this work was to further determine the safety and efficacy of the chimeric PCV1-2b virus in vaccinated pigs that are co-infected by other swine pathogens. Previous work has showed that PCV2 co-infections with porcine parvovirus (PPV) or porcine reproductive and respiratory syndrome virus (PRRSV) enhance PCV2 replication and associated diseases (4). To determine the safety and efficacy of this new chimeric vaccine, we (A) concurrently inoculated pigs with the PCV1-2b vaccine virus and PRRSV and PPV, (B) tested for evidence of chimeric PCV1-2b vaccine virus transmission to non-vaccinated contact pigs, and (C) determined the efficacy of the chimeric PCV1-2b vaccine in a PCV2b-PPV-PRRSV coinfection triple challenge model.

Materials and methods

Forty-six, 21-day-old, PCV2-naïve pigs were randomly assigned to one of six groups according to Table 1.

Table 1. Experimental design.

Group	n	PCV1-2b vaccination		Challenge
		Day 0	Day 28	Day 28
Negative	8	-	-	-
Positive	8	-	-	YES
Vac-0	9	YES	-	-
Vac-0-PCV2	9	YES	-	YES
Contact-PCV2	3	-	-	YES
Vac-28-PCV2	9	-	YES	YES

Vaccination was done intramuscularly for all groups. The contact-PCV2 group was housed together with Vac-0-PCV2 starting at day 3. Blood was collected on a weekly basis and tested for anti-PCV2 antibodies by a PCV2 ELISA (5) and for presence of PCV2 (6) and PCV1-2 DNA by quantitative PCR. At day 28, all pigs were challenged with PCV2b, PRRSV and PPV. Necropsy was conducted at day 49.

Results

One of 8 positive control pigs developed clinical disease consistent with porcine circovirus associated disease (PCVAD) and 3/9 Vac-28-PCV2 pigs developed severe PCV2-associated microscopic lesions. PCV1-2b vaccination 28 days before challenge resulted in significantly ($p<0.05$) decreased amounts of PCV2 viral loads in tissues and sera, and significantly reduced ($p<0.05$) microscopic and macroscopic lesions. PCV1-2b vaccine virus DNA levels remained similar in all vaccinated pigs regardless of the coinfection status. Non-vaccinated contact control pigs (Control-PCV2) became PCV1-2b viremic and were protected against PCV2b challenge**.**

Discussion

The results of this study indicate that the new generation live-attenuated PCV1-2b vaccine remains attenuated, induces protective immunity against PCV2b when administered 4 weeks before PCV2 exposure in growing pigs, and is safe to use in pigs co-infected with PRRSV and PPV. Although the vaccine was transmitted to non-vaccinated contact pigs, those pigs were protected from subsequent PCV2 challenge. The use of the chimeric PCV1-2b vaccine virus to cost-effectively and safely vaccinate large populations of pigs appears to hold great potential.

Acknowledgements

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P.083

EFFICACY OF INGELVAC CIRCOFLEX® IN A RUSSIAN LARGE-SCALE PRODUCTION SYSTEM WITH SUBCLINICAL PCVD

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Introduction

PCV2 vaccination with Ingelvac CircoFLEX® has been shown to improve performance on PCV2 postive farms, even in cases where disease was present in a very mild form, lacking typical clinical signs (1, 2). The goal of our study was to analyse the efficacy of Ingelvac CircoFLEX® on a large-scale Russian pig farm with subclinical PCVD.

Materials and methods

The study was conducted in a large production system (12,000 sows) in Russia. Farm A is a sow farm supplying grow-finish pigs to farm B and gilts to farm C. Farm C is a farrow-to-finish farm, receiving gilts and semen from farm A. All three farms were PCV-2 positive (in ELISA and PCR). Farms B and C were severely affected by PRDC and acute APP at 100-130 days of age, with first clinical signs being observed at 76-80 days. They were positive for EU PRRSV, *M. hyopneumoniae* and APP (seroprofile shown in figure 1). Farm A has a high health status.

In autumn 2009 APP vaccination was introduced to the system. Morbidity and mortality was clearly reduced after APP vaccination, but PRDC was still observed in grow-finishing. In our study (Jun-Dec 2010) piglets were vaccinated with Ingelvac CircoFLEX® one dose (1ml) at 3 weeks of age (VG) or were left non-vaccinated as controls (NVG). All pigs (VG and NVG) were vaccinated twice against APP. Performance of vaccinated pigs was followed on farm A (future breeding gilts) and farm C (grow-finish pigs). Due to management reasons performance data was not available from farm B. Control groups were kept under same management conditions as vaccinated pigs, in different barns on the same site (side-by-side).

Figure 1. Seroprofile of pigs on farm C (Nov. 2009).

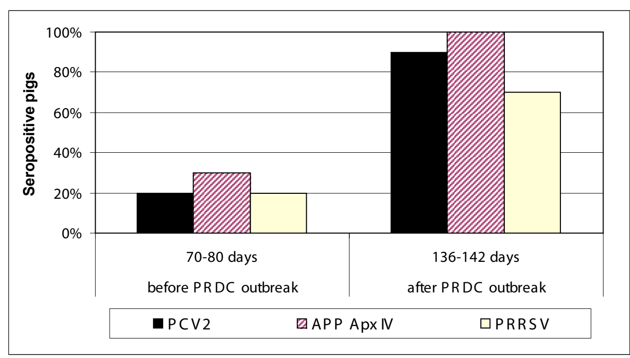

Table 1. Performance results of vaccinated and non-vaccinated future breeding gilts and grow-finishing pigs (Mean = M±S).

Item	Gilts (Farm A)		Finishing pigs (Farm C)	
	VG	NVG	VG	NVG
No. of pens	2	3	5	11
No. of pigs	1,087	849	4,766	11,142
Mean starting age, days	83.0 ±1.0	83.0 ±1.0	99.20 ±3.19	102.36±2.58
Mortality, %	0.28	0.35	1.24	1.62
Cull, %	0.82	1.53	1.43	2.13
Livability, %	98.90	98.12	97.33	96.25
Average daily gain (ADG), g	856.0 ±26.87	799.0 ±78.08	819.80±58.18	784.21±79.16
Mean starting weight, kg/pig	29.0 ±0.83	28.57 ±1.90	43.61 ±1.46	44.65 ±2.76
Mean final weight, kg/pig	103.40±0.52	103.07±6.77	110.42±2.92	109.73±5.44
Total of weight gain, kg/pig	74.53 ±0.50	74.50 ±7.06	66.81 ±4.01	65.08 ±3.18
Term of keeping, days	87.0 ±2.8	93.0 ±2.0	76.4± 3.4	75.5 ±3.0
Mean final age, days	170.0 ±2.83	176.0 ±2.0	175.60±3.36	177.91±3.02

Results

Livability increased 0.78% for gilts and 1.08% for finishing pigs after vaccination. ADG improved by 57 g/day and 35.6 g/day correspondingly. Clinical signs of PRDC and antibiotics costs were considerably reduced among vaccinated pigs. Final weight was bigger and more homogeneous among vaccinated pigs (±0.52 kg and ±2.92 kg vs. ±6.77 kg and ±5.44 kg). Time of keeping for gilts was reduced by 6 days and mean slaughter age decreased by 2.31 days for finishing pigs. Return on investment (ROI) was 2.58:1 for finishing pigs and 3.04:1 for gilts.

Discussion

Results of our study demonstrated good efficacy of Ingelvac CircoFLEX® for subclinical PCVD. Vaccinated gilts and finishing pigs had better parameters than control pigs although evident PCVD was absence. Similar results were described in North America (1, 2). It does indicate that PCV-2 is and important pathogen in PRDC and Ingelvac CircoFLEX is an effective tool to control PCVD even in subclinical disease.

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P.084
THE IMPACT OF PCV2 VACCINATION ON HUMORAL IMMUNE RESPONSE AFTER CSF VACCINATION IN A PCV2 INFECTED HERD

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Introduction

Porcine circovirus type 2 (PCV2) infection can inhibit the humoral immune response of CSFV vaccine [1]. The aim of this study was to assess the feasibility and effect of PCV2 vaccination to ease the immunosuppressive impact of PCV2 infection on CSFV vaccination.

Materials and methods

The study was performed in a pig farm (3000 sows) of eastern China. A total of 820 21-day-old piglets were randomly divided into two groups (418 in vaccinated and 402 in control). Pigs were housed in different barns on the same site. Feeding and management was the same across the groups. Pigs of the vaccinated group were vaccinated with Ingelvac CircoFLEX® on 21-day-old with 1ml dosage. Control group was injected physiological saline on 21-day-old with 1ml dosage. All piglets were vaccinated with CSFV vaccine on 28-day-old with 1ml dosage and 63-day-old with 2ml dosage.

Blood samples were collected from the superior vena cava at 42d, 56d, 70d, 91d and 112d from 24 pigs randomly selected from each group. These specimens were tested by the CSFV ELISA reagent kits (IDEXX). If the blocking rate of sample is greater than or equal to 0.4, the sample can be judged as positive, which means it passes the test; if the blocking rate is less than or equal to 0.3, the sample can be judged as negative; if the blocking rate is between 0.3-0.4, the sample can be judged as suspicious, and needs to be re-tested.

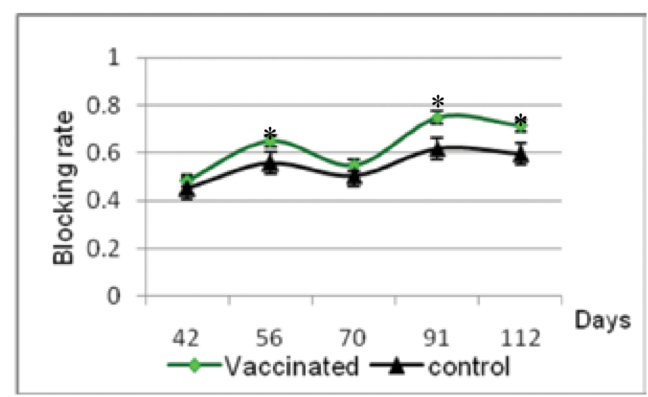
At day 42 and 56 (only controls) serum samples were tested by PCR for PCV2, to evaluate the field infection rate of PCV2 on the farm.

Average blocking rates were compared between the treatment groups using T-test and variance analysis, the percentage of CSFV Ab positive serum samples by Chi-square test.

Results

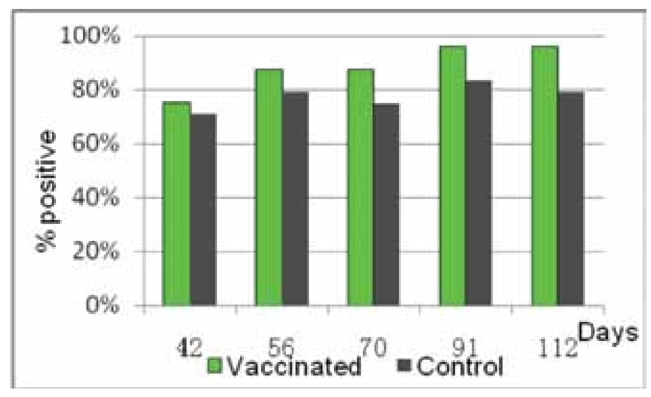
PCV2 infection was confirmed by PCR at both ages (d42 and d56) tested, positive rate is 65.3% (47/72). There was no significant difference of the average blocking rates of CSFV Ab between the two groups at 42 and 70 days old. But at 56, 91 and 112 days old, there were significant differences between the two groups (figure 1). The average variance of the vaccinated group is 0.024; the control group is 0.046, which indicated that the blocking rates of CSFV Ab in vaccinated group are more uniform than that in control group. The percentage (%) of samples positive of CSFV antibodies is shown in figure 2. Overall it was significantly higher in the vaccinated group (87.5% *vs*. 77.5%, p<0.05).

Figure 1. Average blocking rate in CSF Ab ELISA in the PCV2 vaccinated group and the control group.



*Significantly different between vaccinates and controls ($p < 0.05$).

Figure 2. Percentage (%) of samples positive in the CSF Ab ELISA for each sampling day in the PCV2 vaccinated and the control group.



Discussion

It has been shown that PCV2 infection can have a negative impact on the humoral response after CSF vaccination (1). This experiment indicates that Ingelvac CircoFLEX® vaccination might reduce this immunosuppressive influence of PCV2 infection on the humoral immune response after CSFV vaccination.

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P.085

EFFICACY OF INGELVAC CIRCOFLEX® TO IMPROVE NURSERY PERFORMANCE IN A PCV-2 POSITIVE CHINESE FARM

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Introduction

PCV2 was first reported in China in 2000[1]. Since then it rapidly spread to the entire nation and PCVD has become one of the most economically important concerns in Chinese pig farms. The aim of our study was to evaluate the efficacy of Ingelvac CircoFLEX® in nursery performance improvement in a PCVD positive farm.

Materials and methods

The study was conducted on a single-site, 550 sow farm. 82 healthy piglets from 8 litters were randomly divided into two groups. 41 piglets in the vaccinated group were injected with one single dose (1 ml) of the Ingelvac CircoFLEX at 15 days of age while the other 41 piglets in the control group were treated with normal saline (1 ml) at the same time. Management and other vaccination program were the same; both groups were kept in different pens in the same barn. Feed gain ratio (FCR), morbidity, mortality and culling rate were recorded during this trial. Morbidity calculated based on the number of pigs showing clinical signs: high fever, diarrhoea, paleness, cough, laboured breathing, or wasting. Pigs were observed between 15 and 65 days of age.

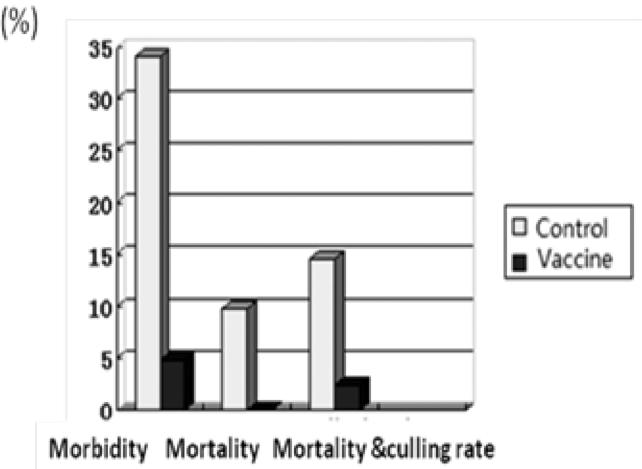
Results

Comparing with the control group, morbidity, mortality and culling rate in vaccinated group were clearly reduced, while the production performance like FCR was also improved. In the control group, morbidity and mortality is respectively 34.1% (14/41) and 9.8% (4/41) while in the vaccinated group the number was reduced to 4.8% (2/41) and 0% (0/41) correspondingly. The mortality and culling rate was reduced from 14.6%（6/41）in the control group to 2.4%（1/41）in the vaccinated group (see Fig.1). Feed gain ratio in the control group is 1.53:1 while in the vaccinated group the feed gain ratio is 1.39:1.

Discussion

Lots of Chinese pig farmers have long been suffering from the high loss in nursery. During this side by side trial of vaccination with Ingelvac CircoFLEX® in a Chinese farm, the performance of vaccinated pigs in nursery was markedly improved comparing to the control pigs, indicating that PCVD problems can be effectively controlled by vaccination with Ingelvac CircoFLEX. The result of this trial showed that vaccination can reduce morbidity, mortality and culling rate in nursery. At the same time, the use of medication was also reduced which suggested that the secondary bacterial infection was reduced by controlling the PCV2. Another benefit is that the feed conversion rate was also improved.

Figure 1. Morbidity, mortality and culling rate of the vaccinated group were reduced comparing to the control group at the age of 65 days



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P.086
COMPARATIVE EVALUATION OF TWO PCV2 VACCINES IN PIGLETS

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Introduction

At present in Europe several commercial PCV2 vaccines (1) are available for use in piglets. One of these is also licensed for sows. Under field conditions, all PCV2 vaccines available are effective, showing decreased mortality and cull rates and significantly improving average daily weight gain (ADWG) (2). The objective of this study was to compare the efficacy of two PCV2 vaccines.

Materials and methods

A controlled, blinded and randomized field study was performed on an Italian 420-sow farrow-to-finish farm without PCVD anamnesis. One thousand and fifty piglets, from five consecutive batches, were included in this trial. The piglets were stratified by sex, weight, parity of the sow and randomly allocated to 3 groups. At weaning, each piglet received 0.5 ml Circovac® i.m. (Group A) or 1 ml Circoflex® i.m. (Group B). The pigs in Group C were not vaccinated and kept as control animals. Control and vaccinated piglets stayed intermingled. All animals were vaccinated against *M. hyopneumoniae* 2 days before PCV2 vaccinations, with a one-shot W/O vaccine.

The body weight was determined at 25, 110 and 225 days of age (d). All animals were monitored for clinical symptoms and mortality was recorded during the whole study period. Only batch 3 was chosen for collection of laboratory data in order to assess the impact of vaccination on viral pressure over time. Blood samples were collected monthly and serum samples of Circovac® vaccinated (n=12), Circoflex® vaccinated (n=13), as well as of control animals (n=11) were analyzed by a competitive ELISA. Pooled sera from at least 3 subjects were analyzed by quantitative PCR (qPCR). The ADWG from 25d to 105d, 105d to 220d and from 25d to 220d in the different groups were compared. The comparison was made by assessing any differences between: a) vaccinated subjects (groups A and B) to group C, b) vaccinated with Circovac® or with Circoflex® to non-vaccinated animals (group C), d) vaccinated with Circovac® to animals vaccinated with Circoflex®.

Results

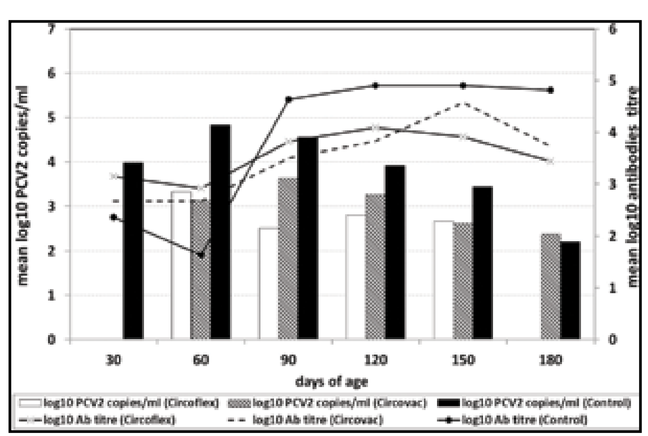
In the periods 25-105d, 105-220d and 25-220d the AWDG of vaccinated subjects were significantly higher (respectively: Mann-Whitney U=97767, $p<0.01$; U=97477, $p<0.05$; U=92101, $p<0.01$) (Table 1) than those of the control animals. Both animals vaccinated with Circovac® and those vaccinated with Circoflex® showed ADWG significantly higher ($p<0.05$) than those of the control group during the three considered periods.

Table 1. Growth performances.

Group	ADWG (g)		
	days of age		
	25-105	**105-220**	**25-220**
C (control)	472.6±73.3	710.5±124.1	613.1±86.1
A (Circovac®)	493.5±69.8	730.0±124.4	633.1±86.1
B (Circoflex®)	494.7±70.5	736.1±111.0	637.0±79.7

The difference between ADWG of group B and group A was not statistically significant for any of the three time periods. The percentage of 25-to-220-day dead pigs and runts: 5.73%, 6.26% and 6.28% for groups A, B and C, respectively, were very similar; however, incidence of underweight pigs was statistically lower in the vaccinated groups. Antibodies titre and serum viral load were statistically lower in vaccinated subjects (Figure 1).

Figure 1. Antibody titres and PCV2 serum viral load



Discussion

Compared to the control group, vaccinated animals showed a significant increase of ADWG. Vaccinated animals had a lower titre of PCV2 specific antibodies and the vaccination seemed to reduce the viral pressure in the facilities. This observation is supported by the qPCR data, which showed, that vaccinated animals had a lower serum viral load. No statistically significant difference in the considered parameters was observed between the two vaccines.

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P.087

IMPLEMENTING PCV2 VACCINATION RESULTS IN REDUCTION OF ANTIBIOTIC USE ON A DUTCH FARROW-TO-FINISH FARM

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Introduction

The antibiotic use in the food producing animals is of a growing concern for consumers, human health care, politicians and retail (1, 2). Also the food producing sector itself is looking for (economical) alternatives for these treatments. The Netherlands is among the countries with the highest antibiotic use in food producing animals in the EU (3, 4). Recently the Dutch government issued the goal of a 50% reduction on the use of antibiotics by 2013 compared to 2009 (5). One of the tools of reducing the usage of antibiotics are preventive measurements such as vaccinations. The objective of this study was to evaluate the effect of a PCV2 vaccination on the antibiotic use under field conditions.

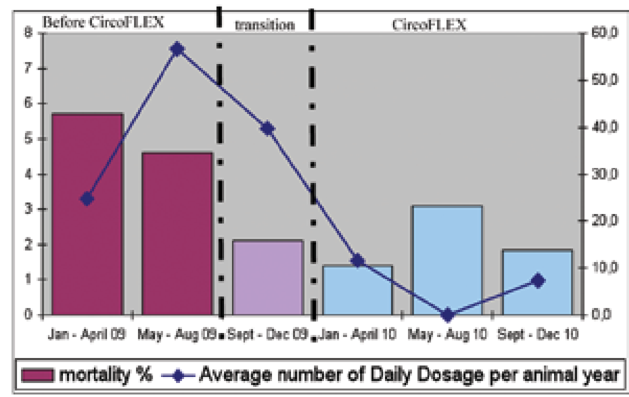
Materials and methods

Production data of a 500 sow farm with 1900 fattening places was retrospectively reviewed for the period January 2009 till December 2010. The fattening unit had a history of diarrhea (Salmonella and Brachyspira negative, Lawsonia positive). Other clinical signs included an in increased number of runts, pigs growing apart, and a high mortality (including euthanasia). There were no lung problems involved. The general treatment was to medicate with tiamulin on a regular basis in the fattening unit. Investigation on blood samples from several runts (mid fattening), showed high levels of PCV2 virus load. In July 2009 the farm started with vaccinating Ingelvac CircoFLEX® (1 ml) at 3 weeks of age. Continuous flow data of the fatteners was used for evaluation. 8 months before vaccination (total of 2869 pigs) were compared to 12 months in which only vaccinated pigs were present on the farm (5933 pigs). The transition period lasted from September to December (1944 pigs) with vaccinated and non-vaccinated being present in the finishing unit at the same time. The parameters mortality and antibiotic use were monitored. For evaluation and comparison of the antibiotic usage in time, the standardized method of Defined Daily Dosage (DDD) of antibiotics used per animal year was applied (4, 6, 7).

Results

The mortality was reduced by 46 % (4,03 vs 2,15%; fig 1), comparing non-vaccinated versus Ingelvac CircoFLEX® vaccinated pigs. For the vaccinated pigs it was not needed to treat them with antibiotics for diarrhea anymore and as a result of this there was a reduction in antibiotic use by 85 % (40,61 vs 6,47 DDD per animal per year; fig 1).

Figure 1. *Mortality (%) and average DDD per animal year in the fattening unit for the 6 periods of 4 months*



Discussion

This retrospective analysis of a Dutch pig farm demonstrates that the use of a 1 dose PCV2 vaccine around weaning can improve performance and improve animal welfare. The health status of the farm and the uniformity of the pigs improved (less runts), so less pigs needed to be transferred to another (younger) compartment or euthanized. These results suggest that there are situations where PCV2 vaccination decreases the use of antibiotics and improve the production and economical performance. Similar findings are confirmed in other reports (8,9).

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P.088

EFFECT OF DOUBLE PCV2 VACCINATION (SOW AND PIGLETS) WITH PORCILIS PCV® ONE SHOT ON PRODUCTION PARAMETERS

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Introduction

Porcine circovirus type 2 (PCV2) vaccines can be applied to sows/gilts and piglets, and have demonstrated to be very efficient to control postweaning multisystemic wasting syndrome (PMWS) (1, 2). It is known, however, that high PCV2 antibody titres in piglets at vaccination may interfere with the serological response elicited by the vaccine (5).

Therefore, the objective of this field study was to assess the effect of both sow and piglet vaccination with a single dose of Porcilis PCV® on productive parameters.

Material and methods

One week before mating, 57 sows from a two-site farm with a previous diagnosis of PMWS were randomly distributed into two groups: vaccinated sows (V, n=26), receiving 2 ml of Porcilis PCV®, and control sows (NV, n=31), receiving 2 ml of PBS.

At farrowing, all healthy piglets (n=476) from these sows were included in the study. Cross-fostering was not allowed. At 3-4 weeks of age (weaning), piglets were distributed into 2 groups taking into account the parity number and the treatment received by the sow. From each sow, half of the litter was vaccinated with Porcilis PCV® (V) and the other half non-vaccinated that received PBS (NV). Therefore, the 476 selected animals belonged to one of these 4 groups: NV sows-NV pigs (NV-NV, n=134), NV sows-V pigs (NV-V, n=135); V sows-NV pigs (V-NV, n=104) and V sows-V pigs (V-V, n=103).

All animals were weighted and scored for physical condition at 3-4, 12, 16, 21 and 26 weeks of age. Average daily gain (ADG) was calculated as the final weight at 26 weeks minus the weight at 3-4 weeks of age divided by the length of the study. Mortality was registered throughout the trial.

Analysis of variance with Bonferroni multiple comparisons and a chi-square test were used to compare weight, ADG and mortality between the four experimental groups. Moreover, the coefficient of variation (CV) was calculated to estimate weight homogeneity at 26 weeks of age.

Results

Wasting was mainly observed in pigs at 16 weeks of age, but PMWS laboratorial diagnosis was not performed. The global mortality rate was lower in V (NV-V=5.2% and V-V=6%) piglets than in NV (NV-NV=8.3% and V-NV=11.5%) ones, but these differences were not statistical significant.

ADG from 4 to 26 weeks of age was 600, 614, 620 and 629 g/day for NV-NV, V-NV, NV-V and V-V piglets, respectively. Statistical differences ($p<0.05$) were observed between NV-NV and V-V groups. Statistical differences in body weight were not observed between groups until 26 weeks of age. At this point, the V-V group showed a significantly higher weight than the NV-NV one. Moreover, weight CV at 26 weeks of age was better for the V piglets (NV-V=10.3% and V-V=8.5%) than for the NV ones (NV-NV=13.8% and V-NV=11.6%).

Discussion

Vaccination with Porcilis PCV® one shot in piglets at weaning was able to significantly improve production parameters and homogeneity of the batch at slaughter age (independently of the PCV2 vaccination sow status) in comparison with non–vaccinated piglets from non-vaccinated sows. These results suggest that although some interference in piglet humoral response development may occur (5, 6), this vaccine is efficacious in terms of improving production parameters under field conditions.

Although the double vaccinated group (V-V) reported the best production parameter results, these were not significantly different from those of vaccinated pigs coming from non-vaccinated sows (NV-V). An economical assessment of these differences would help deciding the most convenient vaccination schedule.

Importantly, it cannot be ruled out the possibility that a later vaccination of piglets coming from PCV2 vaccinated sows, therefore preventing a potential detrimental effect of maternally derived immunity, would have provided even better results.

Acknowledgements

This study was supported by Intervet International BV.

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P.089

SAFETY AND EFFICACY EVALUATION OF DIFFERENT VACCINATION SCHEDULES IN SOWS

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Introduction

Post-weaning multisystemic wasting syndrome (PMWS), PCVD, parvovirosis, erysipelas, neonatal colibacillosis are major diseases in pig farms worldwide. Simultaneous vaccination of the animals is a very convenient way to save labour and to decrease repeated stress. Previous internal trials of simultaneous vaccination (non-published data) with either PMWS/ PCVD (CIRCOVAC® coded CIR, Merial) and Parvovirosis/Erysipelas (PARVORUVAX® coded PAR, Merial) or CIR and colibacillosis (NEOCOLIPOR® coded NEO, Merial), both vaccination schemes being applied during pregnancy, did not rise safety concerns. This paper reports a study having investigated in a larger scale under field conditions in France, the safety of the two simultaneous vaccination schemes in comparison with the recommended schedules.

Materials and methods

Overall 181 sows, coming from 2 different commercial farms usually vaccinated with CIR and PAR were included into the study. In each farm, sows of each farrowing batch were randomly assigned to one of three identical treatment groups according to parity: Group 1 (G1): simultaneous vaccination with CIR,PAR 3 weeks before farrowing, and with NEO 2 weeks before farrowing. Group 2 (G2): simultaneous vaccination with CIRC, NEO 2 weeks before farrowing, and with PAR during lactation. Group 3 (G3) was the control group: CIR vaccination 3 weeks before farrowing, NEO 2 weeks before farrowing and PAR during lactation. These vaccination schemes were implemented during 2 breeding cycles. Systemic and local site reactions as well as rectal temperature were checked during the first cycle on D0, D0+4H, D1, D2 and D7 after each injection. Statistics [1] used ANOVA (or Kruskall-Wallis test if non-normal data) and Mantel Haenszel chi-square test with p<0.05 as level of significance.

Results

The baseline criteria (parity distribution, median parity and rectal temperature at inclusion) were similar in the three groups. Rectal temperatures were compared within physiological periods. First period (P1) was 3 weeks before farrowing (G1 with CIR,PAR vs G3 with CIR), second period (P2) was 2 weeks before farrowing (G2 with CIR,NEO vs G1 and G3 with NEO) and third period (P3) was 2 weeks after farrowing (PAR vaccination, G2 vs G3). The course of rectal temperature is shown in table 1. Depending on the periods, the temperature values were consistently in the normal physiological range for sows: 38.0°C to 38.6°C before and 38.9°C to 39.5°C after farrowing.

Table 1. Adjusted mean rectal temperature (in °C) by group over time

	Group	D0	D0+4h	D1	D2	D7
Period1	G1	38.1	38.5	38.3	38.0	38.1
	G3	38.1	38.4	38.3	38.1	38.1
	Statistics	NS				
Period 2	G1	38.1	38.3[b]	38.2[b]	38.2	38.1
	G2	38.1	38.6[a]	38.4[a]	38.1	38.1
	G3	38.1	38.4[b]	38.2[b]	38.2	38.1
	Statistics	NS	*p<0.001*	*p<0.001*	NS	NS
Period 3	G2	39.0	39.4	39.1	39.0	38.8
	G3	39.0	39.5	39.1	39.0	38.9
	Statistics	NS				

NS: non significant

For P1 and P3, no significant difference was observed between the rectal temperatures of the three groups. For P2, at D0+4h and at D1, a significant higher temperature was observed in the group 2 compared to the groups 1 and 3. However, it was moderate (0.2°C to 0.3°C more) and very transient, temperatures being similar in all groups after D1. On any time point of the study, no significant difference was observed between groups in general condition. No significant difference was observed between the numbers of piglets born alive per sow of the three groups, neither for the first cycle (respectively 13.3, 14.3 and 13.4 in the groups 1, 2, 3), nor for the second one (respectively 12.6, 13.3 and 13.7 in the groups 1, 2, 3). No significant difference was observed between groups in the proportion of sows with local reactions after vaccination.

Discussion

A moderate and transient higher temperature was observed in the group receiving CIR, NEO 2 weeks before farrowing, with no impact on the general condition. These results show the safety and the efficacy of the different simultaneous vaccination schedules since no impairment was observed in born alive piglet.

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P.090
EFFECT OF DOUBLE PCV2 VACCINATION (SOW AND PIGLETS) WITH PORCILIS PCV® ONE SHOT ON PCV2 VIRAEMIA AND SEROLOGY

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Introduction

Nowadays there are 4 commercial vaccines against porcine circovirus type 2 (PCV2) available at the market. Three of them are registered to be applied to growing piglets and the fourth one is an inactivated vaccine to be used in gilts, sows and growing piglets. Field data have demonstrated that these vaccines are efficacious in ameliorating PMWS effects (1).

On the other hand, benefits of double PCV2 vaccination (sow and piglet vaccination) has been only evaluated under experimental conditions (2).

Therefore, the objective of this field study was to assess the effect of double vaccination (sow and piglets) with Porcilis PCV® one shot on PCV2 viremia and serology of piglets.

Materials and methods

One week before mating, 57 sows from a two-site farm with a previous diagnosis of PMWS were randomly distributed into two groups: vaccinated sows (V, n=26), receiving 2 ml of Porcilis PCV®, and control sows (NV, n=31), receiving 2 ml of PBS.

At farrowing, all healthy piglets (n=476) from these sows were included in the study. Cross-fostering was not allowed. At 3-4 weeks of age (weaning), piglets were distributed into 2 groups taking into account the parity number and the treatment received by the sow. From each sow, half of the litter was vaccinated with Porcilis PCV® (V) and the other half non-vaccinated that received PBS (NV). Therefore, the 476 selected animals belonged to one of these 4 groups: NV sows-NV pigs (NV-NV), NV sows-V pigs (NV-V); V sows-NV pigs (V-NV,) and V sows-V pigs (V-V).

Blood samples from 75 piglets in each treatment group were taken at 3-4, 8, 12, 16, 21 and 26 weeks of age. Serum samples were tested by PCV2 antibodies (immunoperoxidase monolayer assay, IPMA) and PCR. Those PCV2 PCR positive samples were tested by quantitative PCR (qPCR) (3).

Results

PCV2 antibody dynamics were different for each of the four treatment groups (Fig 1). Percentage of PCV2 PCR positive serum samples in all piglets from the study are summarised in Table 1. In general, PCV2 viral load was low (<10^6 mean PCV2 copies/ml) throughout the study and was not related with treatment distribution.

Figure 1. Log_2 PCV2 IPMA titres NV-NV (■), NV-V (♦), V-NV (▲) and V-V (●) pigs.

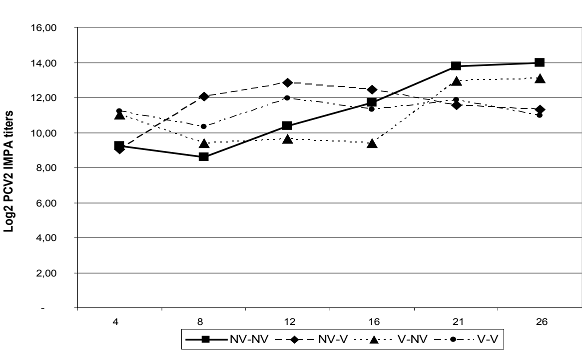

Table 1. Percentage of PCR positive piglets across the study.

	Weeks of age					
Group	**3-4**	**8**	**12**	**16**	**21**	**26**
NV-NV	1,3	11.1	33.8	57.1	55.1	50.0
NV-V	1.3	6.9	13.8	12.5	12.7	2.9
V-NV	1.3	6.9	20.0	37.6	58.8	42.7
V-V	1.3	4.3	1.5	13.0	14.7	5.9

Discussion

Results of this study show that a single vaccination in sows before mating was able to induce high antibody titres to their piglets at 3-4 weeks of age and a delay in PCV2 infection compared to piglets coming from NV sows.

Piglet vaccination (independently of sow treatment) caused an earlier seroconversion and lower percentages of PCV2 infected pigs compared to the NV ones. However, the double (sow and piglets) PCV2 vaccination strategy was able to reduce PCV2 infection and to improve production parameters (6), but apparently caused some interference in piglet humoral response development.

Acknowledgements

This study was supported by Intervet International BV.

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P.091

INFLUENCE OF MATERNAL PCV2 ANTIBODIES ON SEROCONVERSION AND AVERAGE DAILY GAIN IN PCV2 VACCINATED PIGLETS

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Introduction

Porcine circovirus type 2 (PCV2) vaccines have demonstrated to be an excellent tool to control postweaning multisystemic wasting syndrome (PMWS) under field conditions (1). Vaccination of sows and gilts increases PCV2 antibody titres in serum and colostrum, providing protection of piglets against disease development. Piglet vaccines are applied around 2-4 weeks of age and elicit PCV2 total and neutralising antibody responses and reduce and/or delay PCV2 infection (1).

Maternally derived antibody (MDA) interference with piglet vaccine-elicited humoral response has been recently demonstrated (2). However, this concept as well as the potential effect of such interference on productive parameters has not been fully assessed under field conditions. Only one peer-reviewed work has tackled the issue, reaching the conclusion that there is no interference between PCV2 antibody titres at vaccination and pig growth (3).

Therefore, the goal of this study was to further investigate if PCV2 MDA may affect PCV2 piglet vaccination efficacy measured as PCV2 antibody response after its application and average daily gain.

Materials and methods

One hundred and fifty healthy piglets from a multi site farm with previous history of PMWS were vaccinated with 2 ml of Porcilis PCV® at weaning (3-4 weeks of age).

This work was part of a larger study involving PCV2 vaccination in sows and/or piglets (4, 5). Blood samples from piglets at 3-4 and 8 weeks of age were tested for PCV2 antibody titres by means of an immunoperoxidase monolayer assay (IPMA) technique (2). Weight of pigs was recorded at 3-4 (weaning) and 26 (slaughter) weeks of age. Average daily gain (ADG) was calculated as the final weight at slaughter age minus the weight at weaning divided by the length of the study.

Two different linear regression analyses were performed. The first one analysed the relationship between PCV2 antibody titres at vaccination (3-4 weeks of age) and the increment of PCV2 antibody titre at 8 weeks of age. The serological values at 8 weeks of age were chosen for this analysis because reflected mainly the effect of vaccine and not yet natural PCV2 infection (5) on the serological evolution. The second analysis was made between PCV2 antibody titres at vaccination (3-4 weeks of age) and ADG during the whole study period (weaning to slaughter).

Results

PCV2 antibodies at vaccination were negatively correlated (β=-0.099) with the increment of PCV2 antibody 4 weeks post vaccination ($p<0.001$). Thus, the higher the maternally-derived PCV2 antibodies at the moment of PCV2 vaccination, the lower the increment of PCV2 antibody titre after 4 weeks.

On the other hand, no correlation ($p=0.78$) was observed between ADG and PCV2 antibodies at the moment of vaccination.

Discussion

PCV2 vaccine efficacy has been demonstrated in a number of scenarios and also in presence of MDA against PCV2 at the time of vaccination (1). Such efficacy was also observed in the larger study that includes the present work (4). However, since at the time of vaccination it should be expected a variable array of PCV2 antibody levels of maternal origin among piglets, it was important to assess if subpopulations of animals (mainly those with high PCV2 MDA) may have some potential interference with vaccine efficacy.

The present study indicates that the higher the levels of PCV2 MDA at vaccination time, the higher the likelihood of interference with the vaccine-induced humoral response. These results fit with previous experimental data obtained using the same vaccine (2). Importantly, this study shows that this interference at antibody response level was not apparently associated with an effect on ADG, which is one of the most important economical parameters in pig production when evaluating vaccine efficacy.

Acknowledgements

This study was supported by Intervet International B.V.

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P.092
T-CELL IMMUNE RESPONSE IN PIGS INOCULATED WITH INACTIVATED VACCINE AGAINST PORCINE CIRCOVIRUS TYPE 2 (PCV2)

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Introduction

Porcine circovirus type 2 (PCV2) is the causative agent of Post Weaning Multisystemic Wasting Syndrome (PMWS) and other associated diseases (PCVAD). PCV2 has emerged as a major problem in North America and Europe (1-2). The control of PCVAD often depends on minimizing the effect of PCV2. Commercial vaccines against PCV2 are available to reduce reducing the loss attributed to PCVAD (3-4). The objective of this study was to evaluate the clinical and the immune response after vaccination with inacitivated vaccine against PCV2.

Materials and methods

We used 25 hybrid commercial pigs between the age of 1-2 months, which resulted negative to ELISA test for PCV2. The pigs were housed in biosecurity level 3 animal facilities at Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia, Italy. The animals were divided into two groups. Group 1 was composed of 14 animals and they were vaccinated, using intramuscular route (neck muscle) with 1 ml of inactivated vaccine with tiomersale and adjuvant.Group 2 was composed of 11 pigs and were used as control. The animals in Group 1 were immunized ones were observed for 42 days. Temperatures and 10 ml of blood samples in EDTA were also collected from each pig at 0,7,14,21,28,35 and 42 post-vaccination day (PVD). ACK solution was added to each sample to lyse the erythrocytes. Peripheral blood mononuclear cells (PBMC) were washed three times with PBS buffer and incubated for 30 min at 4 °C in the dark with PBS containing a mixture of diluted primary antibodies FITC-antipig CD 3,PE-Cy5 antipig CD4,PE Cy5 antipig CD8, PE antipigCD21, respectively. Then, the cells were sorted in a FACSCalibur Flow Cytometer instrument equipped with BLUE laser 488 nm (Becton Dickinson) using the following strategy. Conditions for forward scatter (FSC) and side scatter (SSC, orthogonal light scatter) were adopted from the literature (5).

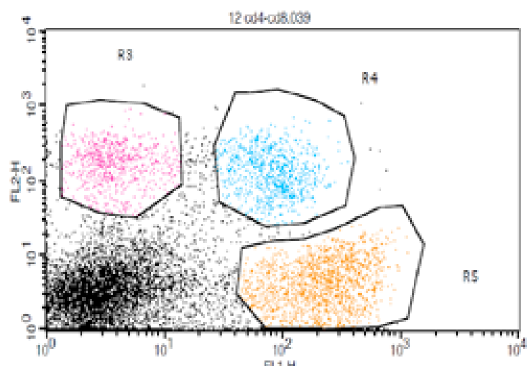
Results

The vaccine did not induce any clinical signs in the immunized pigs during all the period of observation. The rectal temperature was within the normal values and similar to the control values (range 38,5°- 39,2°C). The obtained data from the study of lymphocyte subpopulations involved in the vaccine showed that CD3+ T lymphocytes in the group 1 showed a higher increase in the number of cells than those of control animals. We observed, during the period of vaccination, a stimulation of *naive* lymphocytes and their differentiation into CD4+ CD8-(T helper lymphocytes), and CD4- CD8+ (cytotoxic T lymphocytes). Both the CD4+ CD8+ showed an increase at 14 PVD (Fig.1) and then slowly returned to baseline at 30 PVD. The CD4+ CD8+ subpopulation (memory T lymphocytes) showed an increase in the final period of vaccination, confirming their role as effector of memory cells. Finally, B lymphocytes (CD21+) analyzed in vaccinated animals, did not show a significant change compared to control group.

Discussion

PCV2 vaccination seems to be efficient based on the results from experimental infections and field reports (3). In our study, we described the development of T cell immune response to PCV2 vaccination in pigs. The obtained results are in accordance with those obtained by other (6) using a murine model, they demonstrated that protective immunity against PCV2 in mice was mediated by CD4+ and CD8+ cells.

Figure 1. Plot showing flow cytometry analysis of PBMC.



R: CD4+/CD8- R4: CD4+/CD8+ R5: CD8+/CD4

Acknowledgements

This research was financially supported by the Ministry of Public Health.

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P.093

EFFICACY OF THE MIXTURE OF INGELVAC CIRCOFLEX® AND INGELVAC MYCOFLEX® ON MILD CASE OF RESPIRATORY DISEASE IN SPAIN, LUNG LESION EVALUATION

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Introduction

Vaccination of piglets against *Mycoplasma hyopneumoniae* is commonly practised in combination with PCV2 vaccine to minimize the economic losses caused by both diseases. The effect of both vaccinations is clearly seen when the clinical signs in farm are serious. The efficacy of the recently licensed mixture of Ingelvac CircoFLEX® mixed with Ingelvac MycoFLEX® (FLEXcombo®) to improve weight gain has been shown previously (1).

The objective of this field study was to evaluate the efficacy of vaccination against Enzootic Pneumonia, when it is used combined with vaccine against PCV2 to reduce lung lesions observed in the slaughterhouse.

Materials and methods

A farrow to finish farm with 3-weekly weaning batches and clinical history of mild signs of PCVD and respiratory diseases was selected. The farm suffered from diagnosed mild and chronic problems of *Pasteurella multocida* and *Mycoplasma hyopneumoniae* at the end of fattening phase and sporadic acute outbreaks of *Actinobacillus pleuropneumoniae*. Two batches were vaccinated with one dose of FLEXcombo® 2ml at weaning (3 weeks of age) and data were compared with two batches before the implementation of vaccination.

Lung Lesions was the parameter analyzed in slaughterhouse. Lung inspection was registered individually in 147 non-vaccinated pigs and 200 vaccinated. Lung scoring followed the Madec and Kobisch method (2) from 0-28. During the fattening phase, mortality and runts were registered. Data were analyzed using logistic regression.

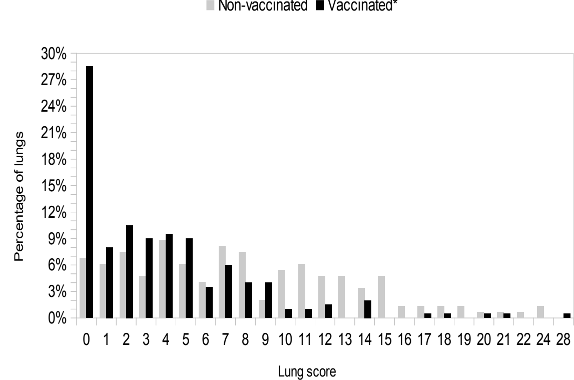
Results

Lung health was significantly improved after vaccination. No lung lesions (score=0) were observed in 28.5% of the vaccinated animals, while in the non-vaccinated pigs 6.8 % had no lesions ($p<0.0001$). Figure 1 shows the percentage of lungs with lesions between 0 and 28 and its distribution among both groups.

Presence of pleuritis was significantly reduced in vaccinated animals, with 23.5% of vaccinated and 46.3% of non-vaccinated pigs having pleuritis ($p<0.0001$).

Mortality rate was similar before and after the implementation of vaccination (3.3% vs 3.0%, $P>0.05$) and percentage of runts was higher in non-vaccinated batches 2.8 % than vaccinated ones 1.3% ($P<0.05$).

Figure 1. Percentage of lungs with lesions between 0 and 28. * FLEXcombo®



Discussion

The results in this field experience show that the use of FLEXcombo® had a significant positive impact on parameters directly related to Mycoplasma infection at the end of fattening. No difference in mortality was observed, as expected in this mild case of respiratory disease.

Lung lesions and pleuritis were significantly reduced in the vaccinated group. The vast majority of vaccinated pigs had no or only very mild lung lesions, ranging between 0 and 5 on the broad scale of the Madec and Kobisch scoring system. In contrast to that, only a few non-vaccinated animals had no lesions and a relevant number had high scores of > 10. The remaining lung lesions in the vaccinated pigs are most likely related to the chronic *Pasteurella multocida* infection occurring in finishing. The *Pasteurella multocida* infection is a continuous problem, as the fattening unit is not working on a complete “all in all out” basis.

During fattening, the non-vaccinated batches needed to be treated two times with doxicicline in water because of an outbreak of respiratory disease. In contrast to that, only a few animals in the vaccinated groups showed respiratory signs and were treated with injectable antibiotics.

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P.094

EFFICACY OF A PCV-2 VACCINE IN AN AUSTRALIAN HERD WITH MILD PCVAD

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Introduction

Porcine circovirus type 2 (PCV-2) has been detected in several herds in Australia (1). It has been shown that vaccination effectively controls PCVAD (2). The objective of this study was to evaluate the effect of a sub-unit PCV-2 vaccine in a herd with mild PCVAD. Ingelvac CircoFLEX® was used at weaning and its efficacy evaluated in terms of growth performance, number of post wean deaths and culled pigs.

Materials and methods

This study was conducted in an 900 sow farrow to finish herd in South Australia. The herd is vaccinating for *M. hyopneumoniae,* the creep and weaner ration has 400 ppm of oxytetracycline and the grower ration 100 ppm of tylosin. An average of 3.5% post weaning mortality and culls (pigs humanely killed before slaughter) was the norm, however certain batches would occasionally be higher. Clinically the only overt signs would be uneven growths. The lung lesion scores ranges from 5.0-6.6%. The consulting veterinarian had implicated the role of PCV-2, based on clinical signs and post-mortem lesions from post transfer to the grower-finisher facilities.

The study involved two batches of weaned pigs in a co-mingled study (Batch 1 = 649 pigs, Batch 2 = 681 pigs, Total N = 1,330 pigs). Piglets were weaned at three weeks of age and were allocated by size and sex into pens. Within each pen, alternate pigs were vaccinated with 1ml of Ingelvac CircoFLEX® vaccine and the remaining pigs left unvaccinated (Control). All pigs were individually identified at weaning and weighed.

Piglets were individually weighed at weaning (3 weeks old), transfer (9 weeks), grower (17 weeks) and pre-market (19 weeks). Any mortalities and culls during the study were recorded. The growth differences were analysed statistically with ANOVA using Statistica v9.0.

Results

Vaccinated pigs in Batch 1 were significantly heavier than control pigs at grower stage and pre-market stage, +1.46 kg and +1.81 kg, respectively (Table 1). However, vaccinated pigs in batch 2, although were numerically heavier, were not statistically significant, +1.02 kg and +0.96 kg.

The data were pooled and the vaccinated animals were significantly heavier by +1.19 kg (p=0.01) and +1.16 kg (p=0.04), at grower and pre-market respectively. The vaccinated pigs grew faster from weaning to pre-market by +11 grams/day than the control pigs (p=0.03).

Table 1. Weights of vaccinated (Vac) and control pigs per batch.

	Batch 1			**Batch 2**		
	Vac	**Control**	**p value**	**Vac**	**Control**	**p value**
No of Pigs	325	324		341	340	
Wean (Kg)	6.14[a]	6.17[a]	0.81	6.16[a]	6.17[a]	0.97
Transfer	27.92[a]	27.91[a]	0.93	30.14[a]	30.72[a]	0.16
Grower	72.79[a]	71.33[b]	0.04	73.85[a]	72.83[a]	0.11
Pre-market	86.67[a]	84.86[b]	0.05	85.57[a]	84.96[a]	0.39

*Different superscripts within the same row and within each batch indicates significance at P≤0.05

Figure 1. Mortalities/Culled from the control & vaccinated groups.

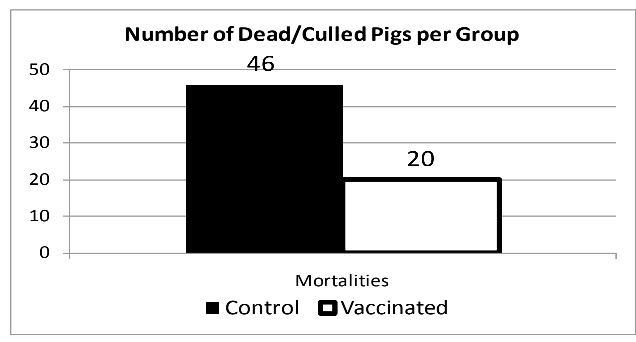

Figure 1 shows the proportion of dead/culled pigs from the control and vaccinated groups. There were significantly more pigs that died or were culled in the control group (6.9%) than the vaccinated group (3.9%, chi-square, p = 0.001)

Discussion

Pigs vaccinated with Ingelvac CircoFLEX® at weaning had a significant growth improvement of +11 gm/day. In addition, the number of deaths and culls were reduced in vaccinated pigs by 43.5%. Lung lesion scoring is being monitored at this time by batches.

Acknowledgements

All veterinary aspects of this study were done under the guidance by Dr. Barry Lloyd.

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P.095

EFFECT OF PORCILIS®PCV VACCINATION ON PRODUCTION AND ECONOMIC PARAMETERS ON A SUBCLINICALLY INFECTED FARM

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Introduction

PCV2 infections are to be found in pigs all over the world. It is well known that PCV2 virus can be detected in apparently healthy as well as in sick animals, and a relationship between PCV2 viral load and the occurrence of disease has been clearly demonstrated (1,2). It is also known that even low levels of PCV2 virus can be significant in having negative effects on production and economic parameters (3).

The aim of this study was to demonstrate the efficacy of PCV2 vaccination on a farm subclinically infected by PCV2, as well as its economic benefits.

Materials and methods

The trial was conducted on a 1,600 sow herd (LDxDuroc crossed with Pietrain) in north-east Spain. The farm is managed on two adjacent sites from which weaned piglets of 14-16kg are moved to fattening facilities located elsewhere. Piglets are always transferred to the same fattening units, which have capacities varying from 1500 to 7500 animals, on a rota system.

The farm is PRRS and Mycoplasma positive. Every four months, all sows are vaccinated against PRRSv (modified live vaccine), and all piglets are vaccinated against *M.hyopneumoniae* at two weeks of age (single dose). No clinical signs indicative of PCV2 infection were observed on the farm, although infection was confirmed by serology (Ingezim PCV2 ELISA®, Ingenasa).

All piglets born after July 2009 were vaccinated with 2ml of Porcilis® PCV between 18 and 24 days of age, a few days before weaning.

The performance of a total of 31,680 vaccinated pigs (11 batches) which entered the finishing units between August 2009 and July 2010, was compared with animals which had been fattened between June 2008 and July 2009 (36,858 pigs in 10 batches) which were considered as negative controls. As there was no significant difference in average weight (p=0.46) and variance of weights (p=0.89) on entry to the fattening units, the groups were considered to be comparable.

Average performance indicators for every batch were recorded throughout the fattening period, including mortality, runts, Feed Conversion Ratio (FCR), Average Daily Gain (ADG), Body Weight at slaughter, Days to slaughter and medication costs.

Statistical analysis was carried out using the Levene test, T-Student test, ANOVA and Pearson's chi-square test.

Results

Table 1 summarizes the production data recorded for the vaccinated and control batches.

Table 1. Production data during the fattening period

	Porcilis®PCV	Unvacc
Animals	31,680	36,858
Nº batches	11	10
Bodyweight on entry	15.21	15.34
Bodyweight at slaughter	105.63	108.32
% Mortality	3.1^{c}	5.6^{d}
% Runts	0.36^{c}	0.96^{d}
FCR	2.69^{c}	2.83^{d}
ADG (g/d)	604.46^{a}	569.17^{b}
Days to slaughter	146.41^{c}	155.81^{d}
Medication costs €	2.86^{c}	3.94^{d}

Values with different superscripts in the same row are statistically significantly different: a, b: p<0.001; c, d: p<0.01

Vaccinated animals had higher ADG than the controls (+42.51g/d; Confidence Interval from 22.35g/d to 62.66g/d) and better FCR (-0.13). Percentage mortality and runts were reduced by 44.65% and 62.5% respectively. In addition, less medication was needed in the vaccinated animals (-1.08€). Although there were no differences in body weight at slaughter between groups, vaccinated pigs showed less variation in weight (p<0.05) and the fattening period was shortened by 9.4 days.

Discussion

An economic model taking account only of improvements in rates of mortality and runts, FCR and medication costs during the fattening period yielded a financial benefit of vaccination of 5.6€/pig, including the cost of the vaccine. Additional benefits, such as better homogeneity in final weights and fewer days to slaughter, should also be noted.

Vaccination with Porcilis®PCV was shown to be an effective tool and a profitable investment, even on farms where PCV2 infection is only subclinical.

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P.096

DUAL STUDY OF THE EFFICACY OF VACCINATION AGAINST PCV2 ON A FARM UNIT WITH A LOW INCIDENCE OF CLINICAL PMWS

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Introduction

When vaccines against Porcine Circovirus first appeared, they were rapidly taken up by farms with major clinical problems. Over time, farms with a lesser incidence of the disease began vaccination, because of the significant benefits to be obtained when the disease is less apparent or even subclinical. This paper describes the data from a comparative study on a farm with a low incidence of the disease designed to investigate the effect of vaccination, together with a comparison of the productivity data before and after the subsequent uptake of a routine vaccine program.

Materials and Methods

The information comes from a farm with 5,100 sows in central Spain, on which animals were positive for PRRSv and *M. hyopneumoniae*. The disease situation was nonetheless very stable, so no vaccination program had been instituted against these agents. The average weight at slaughter was 118 Kg, achieved at about 200 days of age; the mortality rates in the nursery and fattening phases were 1% and 5.2% respectively. The most notable pathology was ileitis at the end of the fattening period. The farm was PCV2 positive, with seroconversion between 12 and 15 weeks of age.

Study 1: Two days before weaning, piglets were randomly assigned to two groups of approximately equivalent composition as to dam of origin, sex and weight, and individually identified. One group of 255 animals was vaccinated with 2ml Porcilis PCV® (ISPAH) at 3 weeks old. The other group, also containing 255 animals, was left unvaccinated, to act as controls.

Study 2: Based on the results of Study 1, it was decided that all the piglets on the farm would be vaccinated according to the regime already described. In this case, productivity in the vaccinated animals (April to August 2010) was compared with that obtained in unvaccinated piglets for the same months in the previous year (April to August 2009).

The statistical tools used were Pearson Chi Squared, Student t test and ANOVA (SPSS 15.0).

Results

Table 1. Results of Study 1

	Control	Porcilis PCV
Average weaning weight (kg)	4.65	4.61 ^{NS}
Average slaughter weight (kg)	109.35	110.45 ^{NS}
Weight gain in fattening phase (kg)	90.23	91.86 *
% losses in fattening phase	2.78	2.36 ^{NS}
% of animals in 1st batch slaughtered	27.57	72.43

Superscripts: NS (not significant): p>0.05; *: p=0.043

The % age of animals in the first batch sent for slaughter gives an indication of the higher ADG and better homogeneity in the vaccinated group of animals.

Table 2. Results of Study 2

	Control	Porcilis PCV
Nº animals	47,245	48,123
Nº batches	19	19
Start weight (kg)	20.48	20.19 ^{NS}
Market weight (kg)	118.5	114.82**
FCR	2.99	2.87 ^{NS}
ADG (g/d)	669	712**
% mortality	5.2	3.4*

Superscripts: NS (not significant): p>0.05; *: p<0.01, **: p<0.001.

In the analysis, correction factors were applied to the start and finished weights for each barn. The resulting differences at the end of fattening are due to differing commercial criteria. In Study 2, when vaccination was applied routinely, because data were available from many more animals, the benefits seen in Study 1 were even more evident, revealing statistically significant differences in ADG, % losses during fattening and an improvement in FCR.

Discussion

Once again, the benefit of vaccination with Porcilis® PCV can be clearly seen, even in the absence of obvious clinical evidence of PMWS.

On this type of farm, the simultaneous comparison demonstrated the possibility of improvement in productivity after vaccination, but the extent of the potential to improve was only revealed following the systematic vaccination of much larger numbers of animals. This is crucial when deciding the type of study to undertake as a function of the starting point.

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P.097
REVEALING OF SUSPECTED VACCINATION FRAUD USING SEROLOGICAL TOOLS

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Introduction

PCV2 piglet vaccination has been shown to be a very effective tool to control PCVD and to improve performance in grow-finish pigs. A lot of finishing farms demand PCV2 vaccinated pigs from their supplier. In some cases pigs purchased as "PCV2 vaccinated" do not perform as expected. It has been shown that evaluation of IgM/IgG antibodies against PCV2 in conjunction with quantitative PCR (qPCR) can help to assess if pigs have been properly vaccinated (1). This case report describes the diagnostic approach in a finishing farm where purchased pigs performed worse than those that were sourced from the own sow farm.

Materials and methods

Seven finisher farms under the same owner with 8600 places receives batches of 1100 pigs from an external farm and 260 pigs from their own sow farm approximately every 3 weeks. Pigs originated from the own sow farm, where vaccinated with Ingelvac CircoFLEX®. The purchased pigs supposed to be vaccinated with Ingelvac CircoFLEX® as well.

Own and purchased pigs are not comingled in the same room (but in the same barn). Pigs are placed on the farm at about 30kg (about 10 weeks of age). During spring 2010 it became obvious, that mortality among the purchased pigs was substantially higher than among the own pigs. The mortality among the purchased batches varied between 4 and 7,6 %, whereas in own pigs it ranged between 1,6 to 2,2 %. Obvious differences were present in the growth. At 16 weeks after placement nearly all own pigs left the farm for slaughter, whereas the purchased pigs remained much longer on the farm, with a significant number still remaining in the farm 18 weeks after placement. Proper vaccination on the supplier farm was questioned. Four batches of purchased were blood sampled as well as five batches of own pigs. From each batch 5 pigs were sampled and tested in INGEZIM CIRCOVIRUS IgG/IgM ELISA. From each batch the 5 samples were pooled and tested by qPCR for PCV2 virus.

Results

The viral load was substantially higher in the purchased pigs compared to own pigs (Fig.1). The lack of IgM response in own pigs during PCV2 exposure clearly shows an excellent priming of the immune system by vaccination, whereas between 80 and 100 % of the purchased pigs had a primary immune response, showing no or insufficient immunization after vaccination (Fig 2). Both own and purchased pigs had an IgG response to PCV2. The IgG reaction in own pigs (data not shown) indicated that virus replication actually took place at week 9 after placement.

Figure1. PCV2 viraemia in own and purchased pigs.

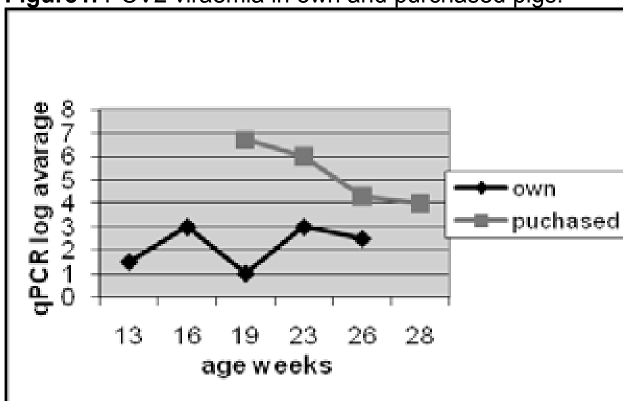
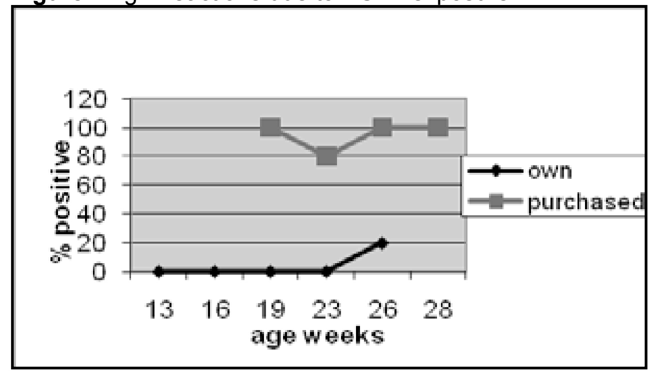


Figure 2. IgM reactions due to PCV2 exposure



Discussion

Confronted with the results, the supplier farm claimed that the pigs were vaccinated with 0.5 ml of a sow vaccine, not with a piglet vaccine. This could explain why the purchased pigs performed worse than the own pigs, as it has been demonstrated earlier that Ingelvac CircoFLEX (1 ml) provides better protection than 0.5 ml of a sow vaccine (2). However, in this case it cannot be excluded that pigs had not been vaccinated at all on the supplier farm.

Acknowledgements

Erling, Lennart og Roger Christiansson

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P.098
A STUDY OF THE EFFICACY OF PORCILIS ® PCV ON A FARM WITH NO CLINICAL EVIDENCE OF PMWS

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Introduction

It is increasingly common to find PCV2 with a moderate or low level of clinical signs. As a result, the control of this disease is more a question of economics. The present study investigates the effects of vaccination on a farm which already enjoys reasonable production levels.

Materials and Methods

A farm with 2,300 sows, in central Spain, was positive for PRRS (seroconversion between 9 and 12 weeks of age) and for *M. hyopneumoniae* (average score: 0.59). It was considered stable for both diseases, so the piglets were not vaccinated. In respect of PCV2, they showed late seroconversion (between 15 and 18 weeks of age) evidenced by 30% of animals remaining IgM positive (Ingezim® Ingenasa) at 18 to 21 weeks old.

At the end of 2009, two animals showing signs compatible with PMWS were sacrificed, and samples taken (lung and tonsils) to look for the presence of the genome of PCV2, but both pigs were negative.

This farm has two very similar fattening units. Without a vaccination PCV2 programme, the performance results were rather variable; for instance, certain barns produced reasonable results in terms of % mortality (2.7%) and FCR (2.5), while others were quite a lot worse (6.55% and 2.84 respectively). In addition, in order to obtain an average slaughter weight of 102 kg and to maintain an appropriate flow of animals (such that a barn could be emptied, washed and disinfected prior to restocking), instead of going to the abattoir, the lower weight pigs (around 80 kg on average) were sent to another barn which had started been emptied

The average percentage of low weight animals was high (21.9%) with a peak of 41.24%. In spite of the absence of clinical lesions and of the viral genome in the tissues of the sacrificed animals, it was decided to vaccinate (single dose of 2ml Porcilis® PCV) at 3 weeks of age, with the aim of improving the situation.

The productivity results from the last barns of unvaccinated animals (fattening beginning January to April 2010) were compared the first barns of vaccinated pigs (fattening beginning May to June 2010).

Statistical analysis was carried out using the Levene Test, Pearson Chi-Squared, Student t Test and ANOVA (GLM, SPSS 15.0 for Windows).

Results

Table 1. Comparison of productivity

	Control	Porcilis PCV
Nº animals	5707	9862
Nº batches	5	9
Body weight on entry (kg.)‡	28.07	28.14 ^{NS}
Body weight at slaughter (kg.)‡	102.14	102.42 ^{NS}
% low weight pigs at end of fattening	21.9	4.8**
FCR	2.71	2.43**
% mortality	4.5	2.3 **

NS: not significant $p>0.05$; **:$p<0.001$.
‡These averages exclude the low weight pigs

It was not possible to analyse ADG properly because the impact of the low weight animals was not known.

There was a considerable improvement in the average % of low weight pigs, FCR and mortality, the worst results in vaccinated animals being 16.03%, 2.51 and 3.71% respectively.

Simply considering FCR alone, with feed costs averaging 0.29 €/kg, this improvement of 280g is equivalent to a benefit of 6.01 € /pig during the fattening phase (from 28 to 102 kg).

Discussion

Vaccination was extremely efficient. The reduction in the number of low weight pigs greatly improved the flow of animals, making emptying easier and reducing labour costs on the farm. As in previous trials, this study has once again shown the benefits of PCV2 vaccination, even on farms on which the pigs show no clinical signs of PMWS.

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P.099

EFFICACY OF PCV2 VACCINATION IN PUREBRED IBERIAN PIGS

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Introduction

PMWS has been clearly linked to PCV2, although it is known that different trigger factors are involved with major and minor outbreaks of PMWS on any given farm (1). Genetics has been considered as one of the numerous risk factors for PMWS. The Iberian pig is a minority indigenous breed in Spain, traditionally reared in extensive or semi-intensive production systems, and much less is known about the effects of PCV2 infection in this breed.

The aim of this trial was to demonstrate that Iberian pigs can suffer from PMWS and that the disease can be very effectively controlled by vaccines.

Materials and methods

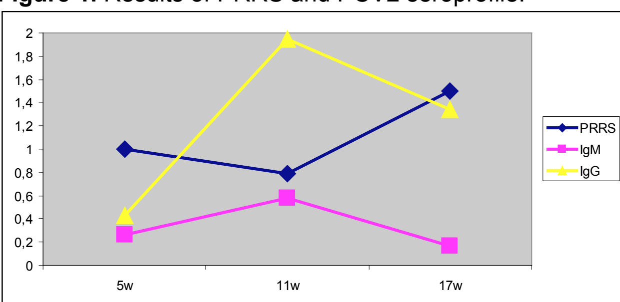
The trial was conducted on a farm with 200 purebred Iberian sows, managed on a two-monthly batch system, with six farrowing batches per year. Piglets are weaned at 35 days of age, and reared semi-intensively up to 50-60 kg bodyweight. Thereafter, pigs are reared together on an extensive system until they are slaughtered at 14-18 months old. This means that production data are only recorded up to 50-60kg bodyweight reached between 18 and 20 weeks old. The farm is positive for PRRSv, *M.hyopneumoniae* and *A.pleuropneumoniae*.

Sows were vaccinated every six months with a live attenuated PRRS vaccine, and, because of a history of respiratory disease during the finishing period, piglets were also vaccinated with the same vaccine at 30 days of age. Piglets also received two doses of a *M.hyo* vaccine in the first and fifth week of life.

The mortality rate in 2008 during the finishing period (up to 60kg) was 5.6%. In 2009, during the same phase, there was a notable increase in mortality due to respiratory disease indicative of PMWS, reaching a peak of 18%. In March 2010, blood samples were taken from animals of 5, 11 and 17 weeks of age. Serum was tested for PRRS antibodies (IDEXX®) and PCV2 IgG and IgM antibodies (Ingezim® PCV2 ELISA). The results are shown in Figure 1. In addition, samples of lung were taken from animals of 14-16 weeks of age, representative of those with overt respiratory disease, and subjected to PCR. They were all negative to PRRSv, *M.hyo* and App. For PCV2, the PCR results were $1.56x10^5$, $7.69x10^4$ and $9.31x10^4$, and it was decided to vaccinate piglets against PCV2. From May 2010 onwards, piglets were vaccinated with 2ml of Porcilis® PCV at 25 days of age. At the same time, PRRS vaccination of piglets was ceased, but *M.hyopneumoniae* vaccination programme was continued.

Statistical analysis was carried out using the Levene test, T-Student test and Pearson's Chi Square test to compare mortality and antibiotic treatment costs between vaccinated and unvaccinated animals.

Figure 1. Results of PRRS and PCV2 seroprofile.



Cut-off +ve values: PRRS >0.4; IgM>0,51; IgG>0.48

Results

Table 1 summarizes performance with regard to mortality and antibiotic medication costs per pig from weaning to 60kg in the vaccinated (batches reared in 2010) and unvaccinated animals (batches reared in 2009).

Table 1. Production data during the fattening period

	Unvaccinated	Porcilis®PCV
Animals	1965	1605
Nº batches	6	4
% Mortality	15.9^a	3.4^b
Medication costs €	2.98	0.96

Different superscript letters in the same row indicates a statistically significant difference: a, b: $p<0.001$

Average mortality was significantly reduced by 78.61% in the Porcilis®PCV vaccinated animals. Medication costs were reduced in 67.8%, though no statistical differences were observed because of the high variance within groups. Also, although these parameters were not recorded in this system, improvements were observed in Average Daily Gain and in the homogeneity of final weights.

Discussion

The use of Porcilis®PCV in purebred Iberian pigs suffering from acute PMWS was shown to be an effective tool for controlling the disease, providing similar improvements in production parameters to those observed in commercial breeds (2).

References

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P.100

COMPARATIVE STUDY OF DIFFERENT COMMERCIAL CIRCOVIRUS VACCINES IN COMBINATION WITH A MYCOPLASMA VACCINE AGAINST A LATE SUB-CLINICAL PCV2 INFECTION

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Introduction

Compatibility of Porcilis® PCV and M+PAC® has been demonstrated in terms of safety and immune response against PCV2 both in the field (1) and *in vitro* (2). It is known that not all PCV2 vaccines produce the same results. The differences between them in controlling viremia mean significant differences in performance (3). The aim of this study was to compare the efficacy of Porcilis®PCV and M+PAC® with that of another commercial combination of PCV2 and mycoplasma vaccine, in terms of productivity and safety.

Materials and methods

The study was performed on a 3-site farm in northern Spain with 2,500 breeding sows, positive for mycoplasma and PRRSV, and a history of 4-6% mortality during the fattening stage. Recirculation of PRRS occurred at 14 weeks of age. PCV2 had been confirmed by clinical signs and gross pathology lesions in 4 of 5 sick pigs euthanized and sero-conversion at 18 weeks of age. 750 piglets were randomly assigned to one of three groups according to their dam, sex and weight: - Group 1: 250 piglets given 2ml Porcilis® PCV and 2ml M+PAC® mixed and injected at a single site; - Group 2: 250 piglets given another PCV2 and mycoplasma vaccine (Vaccine B) in 2ml dose/single site and, - Group 3: 250 piglets injected with 2 ml sterile saline as placebo (controls). All animals were identified, and all doses were given between 18 – 21 days of age. 10 piglets from each group were sampled for PCV2, PRRS and mycoplasma serology at 3, 6, 8,10, 14, 18, 22 and 26 weeks of age, to monitor the dynamics of the infections, using Ingezim PCV2 ELISA®, Ingenasa (IgG,IgM) and an in-house PCV2 antibody ELISA developed by Intervet/Schering-Plough R&D. For weaning and fattening, housing was done in the same barn but with groups allocated in separate pens. All animals were weighed individually at 3, 10, and 26 weeks old (when the first batch was slaughtered), and all individual treatments and deaths were recorded. Linear Method (SPSS 15.0 for Windows) was used for the statistical analysis.

Results

At 3 weeks all animals had an elevated maternal immunity to PCV2, with no significant statistical differences between groups (p=0.597). Group 1 animals sero-converted between 6 and 8 weeks of age (increased titres and 60% positive to IgM) significantly different to the other groups (p=0.001). Groups 2 and 3 sero-converted similarly at 18 weeks (see Figure 1). At the start, no significant differences in body weight were noticed. The same was true (p=0.247) at 10 weeks of age (start of fattening).

Figure 1. Total antibody and PCV2 ELISA results

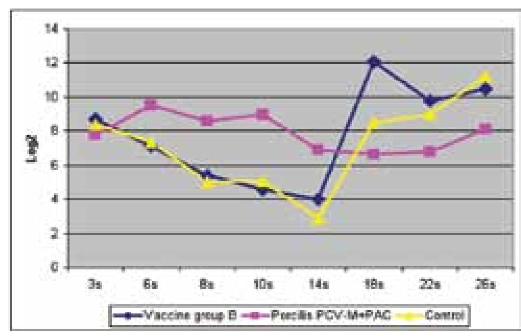

Table 1. Productivity results during fattening period

	Control Group	Group 2 Vaccine B	Group 1 P,PCV/M+PAC
Weight gain (kg)	88.41 [b]	90.5[a]	90.67[a]
%mortality	4.3[a]	3.3[a]	2.5[a]
% treated	33.6[a]	47.6[a]	31.2 [b]
ADG of lightest animals (g/d)	668 [b]	704 [b]	716.3[a]

[a, b,]: values with different superscripts in the same row are statistically significantly different: [a, b]: $p<0.05$

The Porcilis® PCV/M+PAC group had the lowest treatment and mortality rates in absolute terms. Regarding weight, if the animals of each treatment group were subdivided into three weight ranges at the start of the fattening phase (<15.81kg; 15.81-21.1kg; and >21.1kg), then the animals with the best growth by the end of fattening were those which had started at <15.81kg in Group 1 (Porcilis® PCV/M+PAC) (p=0.013).

Discussion

The combination of Porcilis® PCV and M+PAC® leads to appropriate sero-conversion to PCV2 following vaccination. By contrast, the group vaccinated with Vaccine B only sero-converted when it came into contact with the field virus, as did the unvaccinated control group. Not all vaccine combinations produce the same improvements in productivity, Porcilis®PCV with M+PAC® performing better in all the parameters recorded than Vaccine B. Noteworthy, Porcilis®PCV plus M+PAC® was associated to a better growth in the lightweight pigs (compared to vaccine B). It is reasonable to think that most pigs affected by PCV2 probably concentrated there, and the better results were obtained from the more effective vaccine.

References

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P.101

HOMOGENIZATION OF PORCINE CIRCOVIRUS TYPE 2 (PCV2) ANTIBODY LEVELS IN A SOW POPULATION BY MEANS OF VACCINATION

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Introduction

Vaccination of sows and gilts increases PCV2 antibody titres in serum and colostrum, providing protection of piglets against postweaning multisystemic wasting syndrome (PMWS) disease development (1). However, level of PCV2 antibodies at the moment of vaccination in a sow population may be highly variable (2). PCV2 vaccination in sows should be able to homogenise antibody titres to the upper side and, in consequence, deliver a more homogeneous colostrum quality to their piglets.

The main goal of this study was to gain insight into PCV2 antibody and infection dynamics in sows vaccinated with one or two doses of a PCV2 vaccine.

Materials and methods

Seventy seven sows were randomly distributed in two groups: A = sows vaccinated with two doses of Porcilis PCV® (Intervet/Schering Plough) (n=36) and group B = sows vaccinated with one dose of the same vaccine (n=41). Sows from group A were vaccinated one week before mating in two consecutive gestations. Sows from group B were vaccinated one week before mating of the second gestational period under study. Blood samples from these sows were taken at 1st vaccination, at 4 and 11 weeks post-1st vaccination, at 2nd vaccination, and at 4, 8 and 17 weeks post-2nd vaccination. Serum samples were tested by means of immunoperoxidase monolayer assay (IPMA) and by a standard PCR (3).

A non parametric test (Mann-Whitney-Wilcoxon) and a chi-square test were used to compare mean PCV2 IPMA titres and PCR results, respectively, between both groups. Coefficient of variation (CV) was calculated to study the putative homogenization of PCV2 titres in vaccinated animals.

Results

PCV2 IPMA and PCR results are represented in Figure 1. Log_2 PCV2 IPMA titres were significantly higher ($p<0.05$) in A sows at 4 and 11 weeks post-1st vaccination as well as at 2nd vaccination and at 4 weeks post-2nd vaccination compared to B sows. Percentage of PCR positive samples was decreasing over time in both treatment groups, showing no statistically significant differences between groups throughout the study.

CV of both treatment groups for each sampling time is represented in Figure 2. CV was significantly lower ($p<0.05$) in group A than B at 11 weeks post-1st vaccination as well as at 4 and 15 weeks post-2nd vaccination.

Figure 1: Percentage of PCV2 PCR positive serum samples (bars) and mean log_2 (±SD) PCV2 IPMA titres (lines) in A (black) and B (grey) sows.

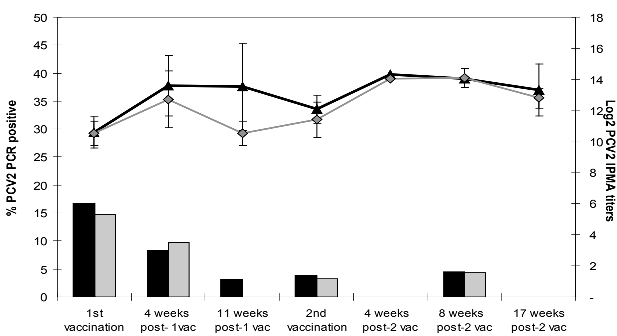
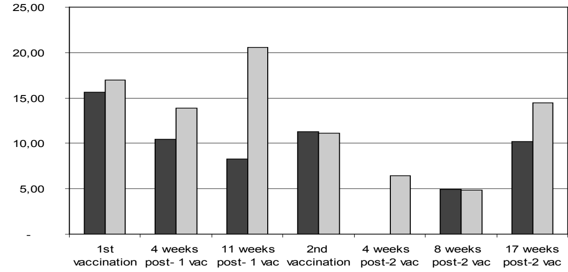


Figure 2: CV (%) of log_2 PCV2 IPMA titres through the study in A (black bars) and B (grey bars) sows.



Discussion

Results of this study suggest that vaccination of sows with Porcilis PCV® elicited a significantly higher and more homogeneous PCV2 antibody titres throughout the 1st gestational period in vaccinated (group A) than in non-vaccinated sows (groups B). Moreover, a second round of PCV2 vaccination increased even more PCV2 antibody titre and homogeneity in both groups of animals. In this scenario, it would be important to evaluate the level of homogeneity of PCV2 antibody titres that piglets get through colostrum intake.

Acknowledgements

This study was supported by Intervet International B.V.

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P.102

META-ANALYSIS OF GLOBALLY PUBLISHED RESULTS ON THE EFFICACY OF INGELVAC CIRCOFLEX® VACCINATION

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Introduction

Porcine Circovirus Diseases (PCVD) are related to PCV2 infection, but many other factors on the farm can affect the severity of the disease and might also affect vaccination results.

Several studies on the efficacy of Ingelvac CircoFLEX® (Boehringer Ingelheim Vetmedica, Inc., St Joseph, MO) have been published since 2007.

The aim of this study was to evaluate the effect of vaccination with Ingelvac CircoFLEX® on mortality in many different studies around the world taking into account some variables related to pig production and to PCVD.

Materials and Methods

This meta-analysis includes 38 trial groups vaccinated with Ingelvac CircoFLEX® (n=41,260) and 38 non-vaccinated groups of pigs (n=38,686) from the same trials. All data evaluated have been published until April 2010.

The effect of vaccination on wean-to-finish mortality has been analysed for the overall and within the following effects: production system (one-site, multisite); infection age (early, medium or late); PRRS status (positive, negative) and trial design, i.e. vaccinated and non-vaccinated pigs comingled in the same pens (yes, no).

The method used for meta-analysis is based on a bivariate approach[1] and it was implemented using the Mixed SAS procedure. Briefly, the analysis includes the information of log-odds for mortality from each trial weighted using estimation error and sample size, and obtaining estimates of log-odds ratio within effects.

Results

Odds ratio mean and confidence interval of treatment is higher than 1 in all groups of the meta-analysis. This indicates a significant positive effect of vaccination in mortality in all trial groups (table 1). In the overall analysis, non vaccinated animals had a 2.75 times higher chance to die than vaccinated ones.

Comparing odds ratio between levels of each effect, two significant effects (i.e. confidence intervals non-overlapped) have been found: the effect of vaccine is clearer (higher odds ratio) in trials with i) pigs in separated pens compared with mixed (comingled), and ii) an early infection age compared with medium infection age (but not compared to late infection age).

Table 1. Differences in mortality and odds ratio between non-vaccinated controls and vaccinated animals.

	Level of effects	Diffe-rence	Odds ratio	Confidence interval Odds ratio
Overall		+5.5	2.75	2.34 - 3.23
Within effects				
Production System	One site	+2.0	2.00	1.61 - 2.48
	Multisite	+5.8	2.65	2.33 - 3.01
	Unknown	+8.9	3.94	2.59 - 6.00
Infection age	Early	+7.7	3.36	2.66 - 4.24
	Medium	+4.4	1.87	1.52 - 2.31
	Late	+3.4	2.17	1.69 - 2.77
	Unknown	+6.8	4.21	3.36 - 5.28
PRRS	+	+9.6	2.99	2.43 - 3.69
	-	+3.8	3.27	2.55 - 4.2
	Unknown	+3.2	2.13	1.68-2.70
Pigs comingled	No	+8.2	3.71	3.16- 4.35
	Yes	+2.9	2.04	1.59 - 2.62

Discussion

The meta-analysis yielded a significant reduction of mortality in Ingelvac CircoFLEX® vaccinated compared to non-vaccinated animals for all different levels of effects, demonstrating the consistent efficacy of vaccination. Vaccinated animals overall had 5 points of percentage of mortality less than non-vaccinated, a reduction of 50%. Further evaluation of this data is needed in order to analyse factors influencing the differences within the effects.

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P.103

THE ECONOMIC BENEFIT OF VACCINATION AGAINST PORCINE CIRCOVIRUS TYPE 2 BASED SOLELY UPON AVERAGE DAILY GAIN IMPROVEMENT; A SUMMARY OF 8 PUBLISHED US TRIALS

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Introduction

Commercial Porcine Circovirus type 2 (PCV2) vaccines have been found to be effective and economically valuable (1). The objective of the present analysis is to estimate the economic benefit of improved average daily gain (ADG) resulting from the use of commercial PCV2 vaccine compared to non-vaccinated contemporary controls across 8 controlled US field studies.

Materials and methods

Eight previously published US-based field trials were included in this analysis. Since both clinically affected (2,3,8,9) (significant difference in mortality rate between vaccinates and non-vaccinates) and subclinically affected (4,5,6,7) (no significant difference in mortality rates) herds were included in this analysis, ADG was selected as the sole biologic parameter for use in estimating the partial economic benefit of vaccination. The experimental unit for calculating ADG varied between studies and was either individual pig (2,3,5,6,9), pen (4,7) or barn (8). In each case the vaccinated group received a single dose of PCV2 vaccine (Ingelvac CircoFLEX®, Boehringer Ingelheim Vetmedica Inc, St Joseph Missouri USA) when weaned at approximately 3 weeks of age per approved labeling.

A temporally matched (experimental unit, number of experimental units, season, pig age and location) non-vaccinated group of pigs was used for comparison. In each individual study the difference between vaccinates and non-vaccinates ADG was assessed by least square means and the P value for the difference in ADG was less than or equal to 0.05.

A net benefit of vaccination was calculated for each study using standardized economic assumptions including: 2.5 nursery-finish feed conversion ratio, USD$0.217/kg feed cost, USD$1.10/kg live weight value for pigs. The mean net benefit values from each individual study were then used to derive a summary mean net benefit and 95% confidence interval across the 8 studies.

Results

The mean net benefit and 95% confidence interval based solely upon significant improvement in ADG across the 8 studies were USD $5.11 +/- $1.97 respectively per vaccinated pig (Table 1).

Table 1. Economic benefit of PCV2 piglet vaccination in 8 published US field trials based solely upon improvements in average daily weight gain.

	N		ADG Ä P value	Net benefit per pig vaccinated (USD)
	Vax	Non-vax		
Study A (2)	374	368	P=0.001	$7.14
Study B (3)	491	483	P=0.0001	$5.84
Study C (4)	40	40	P=0.01	$2.59
Study D (5)	600	600	P=0.0001	$3.24
Study E (6)	493	492	P=0.05	$3.24
Study F (7)	13	12	P=0.05	$6.49
Study G (8)	44	38	P=0.0001	$3.24
Study H (9)	330	326	P=0.04	$9.08
Summary net benefit & 95% confidence interval				$5.11 +/- $1.97

Discussion

Vaccination with a single dose of Ingelvac CircoFLEX® at weaning consistently resulted in significant improvements in biologic and estimated economic performance. Based solely upon ADG improvement, a mean net benefit and 95% confidence interval of USD $5.11 +/- $1.97 was calculated per pig vaccinated across the 8 studies. While not included in the present economic evaluation, additional benefits in reduced mortality (1), improved feed efficiency (8) and carcass traits (5) have been reported with the use of this vaccine.

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P.103B

HAPTOGLOBIN SERUM LEVEL DYNAMICS IN PORCINE CIRCOVIRUS TYPE 2 (PCV2) VACCINATED AND NON-VACCINATED PIGLETS

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Introduction

Porcine circovirus type 2 (PCV2) vaccines have demonstrated to be very efficient to control postweaning multisystemic wasting syndrome (PMWS) and to improve production parameters under field conditions (1,2). Acute phase proteins (APPs) are a group of blood proteins that change their concentration in animals subjected to external or internal challenges such as infection, inflammation, surgical trauma or stress (3)., Haptoglobin (Hp), a positive APP, has been recently claimed as an index for monitoring productive performance. In particular, low serum Hp levels have been correlated with better production parameters in pigs (4).

The objective of the present study was to describe the evolution of Hp serum levels in a population of pigs vaccinated and non-vaccinated against PCV2.

Materials and methods

Three hundred pigs, from a farm with a recent PMWS history, received one single dose of 0.5 mL of CIRCOVAC® at 3 weeks of age (5). As controls, 300 animals received the same amount of PBS. Fifty pigs from each group were bled at 3, 6, 10, 14, 17, 20, and 23 weeks of age.

All animals were weighted at 3, 10 and 23 weeks of age. Average daily gain (ADG) between 3 and 23 weeks of age was calculated. Mortality was registered and all dead pigs were necropsied and analysed for potential PMWS diagnosis (histopathology and PCV2 in situ hybridization).

PCV2 load in sera was investigated by means of a real-time quantitative PCR (qPCR) (6). Hp was quantified in sera by using a spectrophotometric method as previously described (4).

Percentage of qPCR PCV2 positive pigs between groups were compared using a chi-square test. Moreover, Kruskal-Wallis and Mann-Whitney tests were used to compare serum haptoblobin concentration between vaccinated and control animals in each sampling time.

Results

Wasted pigs were observed at the time of higher percentage of qPCR PCV2 positive pigs (17 weeks of age) but clinical signs, at population level, were mild. Recorded mortality was lower in vaccinated (3.9%) than in control animals (5.9%) but statistical significant differences were not observed. PCV2 prevalence in sera was below 5% from 3 to 14 weeks of age, and increased progressively, reaching a maximum at 17 weeks of age in both vaccinated and non-vaccinated groups. Percentage of PCV2 qPCR positive piglets was significantly higher in non-vaccinated pigs versus vaccinated ones at 17, 20 and 23 weeks of age.

Average serum Hp concentration was low (less than 0.7 mg/dL) from 3 to 10 weeks of age and this concentration increased afterwards, reaching a maximum value at 17 weeks of age (1.2 mg/dL) and declining slightly until the end of the study (0.8 mg/dL) in vaccinated and non-vaccinated pigs. Hp values were always higher in non-vaccinated animals than in vaccinated ones from the beginning of PCV2 circulation (14 weeks of age). Such differences were statistically significant at 17 weeks of age, coinciding with the maximum percentage of viremic animals in the non-vaccinated group (90%) compared with the vaccinated one (25%).

ADG was significantly higher in vaccinated (520 g/day) versus control animals (510 g/day) when the whole rearing period was taken into account.

Discussion

Vaccination with CIRCOVAC® in piglets under field conditions was able to significantly improve production parameters and reduce significantly PCV2 viremia (5). These results agree with other studies using piglet vaccination in PMWS affected farms, confirming that production parameters should be considered as good criteria for PCV2 vaccine efficacy. On the other hand, Hp serum concentration profile was different between vaccinated and non-vaccinated pigs suggesting that this APP could be used as an unspecific parameter to evaluate PCV2 vaccine efficacy.

Acknowledgements

This study was supported by Merial Laboratorios S.A (Spain).

CIRCOVAC® is a registered trade mark of Merial in Spain and elsewhere.

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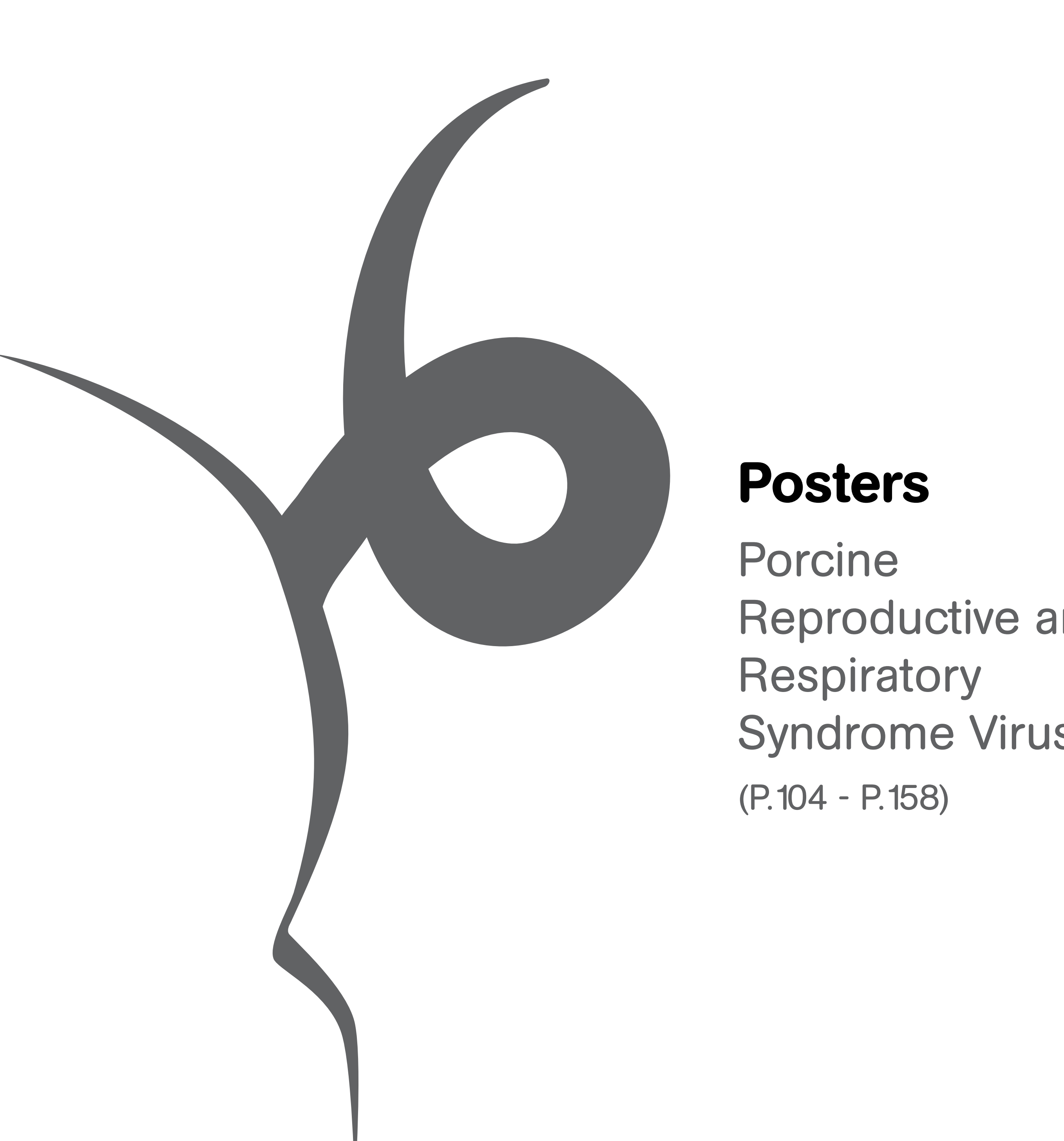

Posters

Porcine Reproductive and Respiratory Syndrome Virus

(P.104 - P.158)

P.104

ESTIMATING THE MEDIAN INFECTIOUS DOSE (ID50) OF PRRSV ISOLATE MN-184 VIA AEROSOL EXPOSURE

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Introduction

The topic of aerosol transmission of porcine reproductive and respiratory syndrome virus (PRRSV) remains an area of active discussion and on-going investigation. As previously described (1, 2), PRRSV transmissibility via aerosol can be quantified by aerosolizing virus into a reservoir, exposing animals to a specific dose, and determining the proportion of animals that become infected by exposure dose. The median infectious dose (ID_{50}), i.e., the dose at which 50% of those exposed become infected, is a useful summary statistic of this dose-response. Likewise, ID_{50} provides the means to compare infectivity among different isolates and/or routes of exposure. Previously, the ID_{50} of airborne PRRS virus isolate VR-2332 was reported as $1 \times 10^{3.1}$ $TCID_{50}$ (Hermann et al., 2009). The objective of this study was to estimate the ID_{50} of the PRRS virus isolate MN-184 via aerosol exposure and compare to the earlier ID_{50} estimate for isolate VR-2332.

Materials and methods

The study was conducted in 10 replicates, with pigs randomly assigned to exposure dose. In each replicate, 8 or 9 pigs were sequentially exposed to successively lower doses of airborne PRRS virus isolate MN-184. Airborne PRRSV for pig exposure was created by nebulizing PRRS virus into a 400 liter stainless steel dynamic aerosol torrid (DAT) rotating at 4 RPM. Virus infectivity was maintained by housing the entire apparatus within a custom-built refrigeration unit held at -4˚C.

Following exposure, animals were individually housed in hepa-filtered isolation units equipped with air, feed, and waste handling systems that maintained a biosecure environment. Serum samples collected from each pig 5 and 10 days post-exposure (DPE) were tested for PRRSV to determine whether exposure resulted in infection. The probability of infection by exposure dose was modeled on the proportion of pigs that became infected by dose. The generalized linear regression models (logit and probit link functions) were used to fit the binary response (infection) with the explanatory variable (exposure dose).

Results

Among all pigs (negative controls and principles), mean body weight on DPE 0 was 12.8 kg (range 10.2 to 17.5 kg). Among aerosol-exposed pigs, the mean time to respire 10 liters of PRRSV aerosol was 5 min and 6 sec. No mortality occurred during the monitoring period, nor was not possible to visually determine which pigs had become infected with PRRSV, i.e., no overt clinical signs were observed.

Data from 3 replicates were excluded from the analysis due to non-compliance with quality control parameters. Based on the remaining 7 replicates, the ID_{50} for PRRSV isolate MN-184 was conservatively estimated as $1 \times 10^{0.26}$ TCID50 (95% CI $1 \times 10^{-0.96,\ 1.09}$).

Discussion

This research found that PRRSV isolates MN-184 and VR-2332 differed greatly in their infectivity via aerosol exposure. Both estimates were produced in the same laboratory working with pigs of approximately the same age and using the same experimental design, equipment, and protocols. Differences in infectivity among isolates from the same species has been reported previously, e.g., Sellers and Gloster (2007) reported that infectivity differed both between FMDV types (O, A, C) and among isolates within types. However, this is the first report of this phenomenon in PRRSV. Overall, the results suggest the existence of a distribution of ID_{50}s among PRRSV isolates for airborne exposure; a distribution for which we currently have only two ID_{50} point estimates.

Acknowledgements

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P.105

PRRSV EFFICIENTLY REPLICATES IN FETAL IMPLANTATION SITES AND CAUSES APOPTOSIS IN INFECTED MACROPHAGES AND SUROUNDING CELLS

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Introduction

Reproductive failure due to PRRSV is characterized by late-term abortions, early farrowing, increased number of dead and mummified fetuses and weak-born piglets. The mechanism of PRRSV-induced reproductive failure is poorly understood. Human pregnancies, complicated by some pathogens leading to reproductive disorders exhibit increased apoptosis in the fetal membranes (1). Therefore, we hypothesized that PRRSV can replicate and induce apoptosis in endometrium/fetal placentas. In the present study, localization and quantification of the PRRSV-positive and apoptotic cells were performed in the fetal implantation sites.

Materials and methods

Three dams were inoculated with PRRSV at 90 days of gestation, euthanized at 10 days post-inoculation and sampled (blood, uterus with placenta corresponding to every fetus; fetal blood and internal organs). Two non-inoculated dams served as control animals.

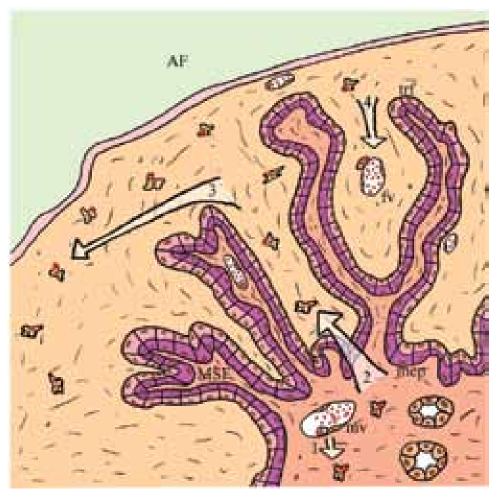
Results

Inoculation of the dams resulted in viremia that lasted until the end of the study. Transplacental PRRSV spread was detected in all inoculated dams. At 10 dpi, 18 (40%) of the 45 fetuses were viremic. Fetal sera from all non-inoculated dams remained PRRSV-negative. Using immunofluorescence staining, single PRRSV-positive cells were found in the endometrial connective tissues of all fetuses. In the fetal placental mesenchyme of the PRRSV-positive fetuses, infected cells were abundant and spread focally. The PRRSV-positive cells between endothelial cells of the maternal blood vessels and close to the fetal placental blood vessel were observed. The number of PRRSV-positive cells in the fetal placentas (0-289/10 mm^2 of tissue) was significantly higher than that in the endometrium (1-16/10 mm^2 of tissue; p=0.004). Double staining showed that all PRRSV-positive cells in the fetal implantation sites were macrophages positive for sialoadhesin and CD163. Apoptotic cells (TUNEL+) were detected in the fetal implantation sites of both non- and PRRSV-inoculated dams. The amount of the apoptotic cells was significantly higher in PRRSV-positive endometrium and fetal placentas (p=0.04 and p=0.001). In the endometrium, apoptotic cells were located within the connective tissues. In the fetal placentas, apoptotic cells were spread in the mesenchyme. In a few sections, apoptotic cells were spaced within the trophoblast layer. A spatial correlation between the sites of PRRSV replication and TUNEL+ cells was observed. Double-labeling revealed that 9 to 57% of the apoptotic cells in the fetal placentas were PRRSV-positive. Within the population of PRRSV-infected cells, the percentage of apoptosis was ranging between 20 and 61%.

Discussion

The maternal viremia leading to PRRSV replication in the endometrium with subsequent fetus infection through the fetal placenta is the likely way of PRRSV vertical transmission (Figure 1).

Figure 1. Hypothetical model of the PRRSV replication in the fetal implantation sites. MSF: maternal secondary fold; mv: maternal endometrial vessel; fv: fetal placental vessel; mep: maternal epithelium; trf: trophoblasts; cells with red crosses represent PRRSV-infected macrophages. 1. PRRSV replicates in susceptible intravascular macrophages adhering to the endothelial cells of the endometrial vessels. Extravasation of the infected intravascular macrophages and PRRSV replication in the endometrial macrophages; 2. After replication in the endometrial macrophages, PRRSV crosses the maternal epithelium and trophoblasts, probably with maternal macrophages that spread to fetal placenta; 3. Focal, highly efficient PRRSV replication in the fetal placental macrophages; 4. PRRSV reaches fetal internal organs most likely through fetal blood, as a free virus or in association with macrophages.



PRRSV infection and/or PRRSV-induced apoptosis of macrophages in the fetal implantation sites may trigger events that finally affect the fragile “immunological constellation” and induce reproductive pathology.

In conclusion, PRRSV efficiently replicates in the fetal implantation sites and causes apoptosis in infected macrophages and surrounding cells.

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P.106

COMPARATIVE PATHOGENICITY OF TYPE 1 AND TYPE 2 ISOLATES OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) IN THE RESPIRATORY MODEL

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is highly variable from a genetic point of view. This fact has led to the division of isolates into two different genotypes: type 1, which comprises European isolates and type 2, which includes American isolates. There are field evidences that indicate that type 1 viruses are mainly involved in reproductive failure while type 2 viruses cause severe respiratory disease in growing pigs (1). However, this assumption has never been experimentally proven. Consequently, the objective of this study was to compare the pathogenicity of three American-Type and three European-Type PRRSV isolates in a respiratory pig model to determine whether there are differences in the virulence of both genotypes.

Materials and methods

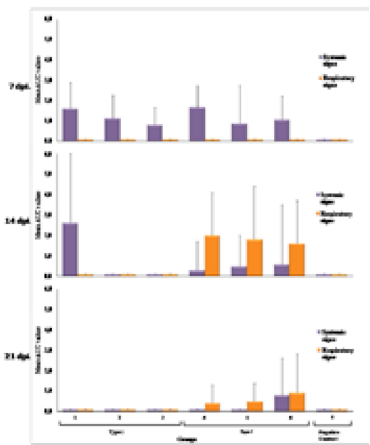
One-hundred 3 week-old piglets were divided into 7 groups. Pigs of groups 1 to 3 were inoculated intranasally with type 1 isolates and pigs of groups 4 to 6 with type 2 viruses. Group 7 was kept as negative control. All pigs were examined daily for clinical signs and serum samples were collected every three days. Five piglets per group were sacrificed on days 7, 14 and 21 post-infection and different tissue samples collected. At necropsy, gross lung lesions were evaluated and samples of lung collected to determine microscopic lesions and PRRSV antigen by immunohistochemistry (2). Viremia and virus organic distribution were determined by virus isolation (3).

Results

Systemic clinical signs, including rough hair, depression, and occasionally lethargy and anorexia, were observed in pigs of all inoculated groups. However, no significant differences were found between groups. On the contrary, respiratory clinical signs(dyspnea, tachypnea and laboured breathing) were only observed in pigs exposed to type 2 PRRSV isolates (Figure 1). Besides, pyrexia was more frequently recorded in pigs exposed to type 2 viruses than in those exposed to type 1 isolates.

Gross lung lesions, characterized by multifocal tan-mottled areas of pneumonia, were observed in all inoculated groups. However the mean percentage of lung surface affected was generally higher in pigs exposed to type 1 isolates. In the same way, microscopic lung lesions were more severe in pigs exposed to type 2 viruses. All pigs exposed to PRRSV were viremic during the whole experimental period and no differences were observed between groups in relation to viral load in serum samples. In the same way, organic distribution of PRRSV and viral load in different tissues was similar in all PRRSV-exposed groups, regardless of the isolate used to inoculate the piglets.

Figure 1. Mean area under the curve (AUC) of clinical signs recorded each week after inoculation.



Discussion

Results of this study show that PRRSV isolates differ in their pathological properties. Particularly, type 2 viruses seem to be more pneumovirulent than type 1 isolates, based on the more severe respiratory clinical signs as well as more severe gross and microscopic lung lesions in pigs exposed to type 2 viruses. However, these pathological characteristics were not related to a higher viral replication since virus organic distribution and viral load in serum and tissues were similar for all inoculated groups, regardless of the infecting isolate. Other pathological mechanisms, as higher production of proinflammatory cytokines by type 2 viruses, might explain the occurrence of more severe respiratory distress in pigs exposed to those isolates.

Acknowledgements

We want to thank Dr. Lars Larsen and Dr. Fernando Osorio for providing the American-type isolates.

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P.107

COMPARISON OF THE PATHOGENESIS OF DIFFERENT EURPOPEAN PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS STRAINS

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) causes respiratory disease and reproductive losses in pigs. The control of infection is difficult due to the quickly changing genetic and antigenic structure of the RNA virus and the appearance of new virus variants, and the insufficient protection by vaccination. In addition, highly virulent strains have emerged that led to high losses.

The objective of this study is to compare pathogenetic mechanisms of different European PRRSV strains in order to understand differences in virulence. More specifically, to define if there are differences in target cells that become infected and to dissect the immunological host responses of pigs infected with different strains

Materials and methods

An animal trial was performed with 4 groups of sixteen pigs. In group 1, pigs were infected with EU type PRRSV strain Lena, known to induce clinical signs. In group 2, pigs were infected with the recently isolated EU type strain Belgium 07V063, that causes subclinical infections. In group 3, pigs were infected with the EU reference strain Lelystad (LV), that also causes subclinical infections. Group 4 were control pigs. At days 7 and 21 post inoculation (p.i.), 4 pigs per group were vaccinated with a pseudorabies (PR)vaccine to study the immune competence of pigs after PRRSV infection.

Weekly, serum was collected for antigen detection and antibody responses, and peripheral blood mononuclear cells were isolated for IFN-y ELISPOT assay and FACS analysis. With FACS analysis, kinetcs of the haematological changes were studied, focusing on the identification of the lymphocyte sub-populations.

At days 3 and 7 p.i., 4 pigs per group were euthanized for post-mortem examination. At day 35, 8 pigs per group (4 vaccinated and 4 non-vaccinated) were euthanized for post-mortem examination. Several tissues were collected for immuno-pathological analysis, to reveal PRRSV load and changes in leukocyte number and composition in tissues.

Results

Immunopathological analysis, PR serology, virus isolations and PCRs are currently performed and results will be presented during the conference. Preliminary data show that the animal trial resulted in the expected clinical pictures, with the Lena strain causing fever and respiratory symptoms, while the Belgium and LV strain caused subclinical infections.

All pigs inoculated with the Lena, Belgium or LV strain were infected and developed antibodies, detectable from 10 days p.i. Virus could be isolated in serum from the Lena and Belgium infected pigs between days 3-33 p.i, and from LV infected pigs between days 3-26. All control pigs remained uninfected.

FACS analysis showed differences in cell populations between the infected and control pigs (NK cells and γδ T) and between strains (B cells and CD4 memory T).

The IFN-y ELISPOT assays showed an PRRSV specific IFN-y response at day 26 p.i., with the highest number of IFN-y secreting cells from Belgium infected pigs, followed by LV infected pigs and the lowest number by Lena infected pigs.

Discussion

The study confirmed the higher virulence of the Lena strain. It is hypothesized that this more virulent Lena strain will infect more cell types of the monocytic cell lineage than the low virulent Belgium and LV strains. Based on the preliminary results, it can already been concluded that there are differences in pathological and immunological host responses between the Lena strain on one hand, and the Belgium and LV virus strain on the other hand

Acknowledgements

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P.108
PRRSV (TYPE I) CHALLENGE IN FINISHING PIGS

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Introduction

A recently isolated PRRSV Type I, subtype 1 field strain has been shown to induce clinical signs and lung lesions upon experimental challenge in growing piglets (1). Since the susceptibility of pigs to PRRSV type II infection is age dependent (2), we wanted to evaluate the pathogenicity of the PRRSV type I isolate in finishing pigs.

Materials and methods

Twenty finishing pigs derived from a PRRSV and *Mycoplasma hyopneumoniae* negative herd were included in the study. At 24 weeks of age, ten pigs were challenged with 2.2 × 10^6 $TCID_{50}$ of the PRRSV type 1, subtype 1 field isolate (challenge group), which has been described to cause clinical signs and lung lesions in growing piglets; ten pigs were not infected and served as control. Viraemia and antibody response to the virus were monitored at 4, 10, 15 and 21 days post infection (DPI). Clinical signs and rectal temperature were monitored throughout the entire experiment on a daily basis, starting 2 weeks prior to challenge. Body weight was recorded 2 weeks before challenge, at the day of challenge, and at 10 and 21 DPI. Half of the study animals were euthanized at 10 DPI and the other half at 21 DPI. The extent of the macroscopic lung lesions (tan mottled areas) and the extent and severity of the microscopic lung lesions were scored. The histopathological score (maximum score: 210) was obtained by summing the severity and extent score of 5 different histological parameters for each lung lobe.

Results

All infected animals became viremic within 4 days after challenge, and 60 % of the pigs were still viremic at the end of the study. Peak virus titer was reached at 4 DPI with 5.8 $\log_{10}$ GE/ml. Seroconversion started at 10 DPI when 80 % of infected animals were positive by ELISA. Significant differences in the mean rectal temperature between the two groups were found at 1, 2, 3, 6, 8, 9 and 10 DPI (figure 1). The peak in rectal temperature was reached at 3 DPI when 60 % of the infected animals had hyperthermia (rectal temperature ≥ 40°C). Within the first 10 days following challenge, infected pigs had a significantly lower body weight gain compared to control animals (figure 2). The median lung lesion extent of challenged animals was 11.4% 10 dpi, and the lesions were more severe at 10 DPI than at 21 DPI. The control group did not show any macroscopic lesions. Similarly, the histopathological score from infected pigs at 10 DPI (mean total score of 105) was higher than the one at 21 DPI (mean total score of 73.4).

Figure 1. Mean rectal temperature for each study group before and after challenge; * $p < 0.05$.

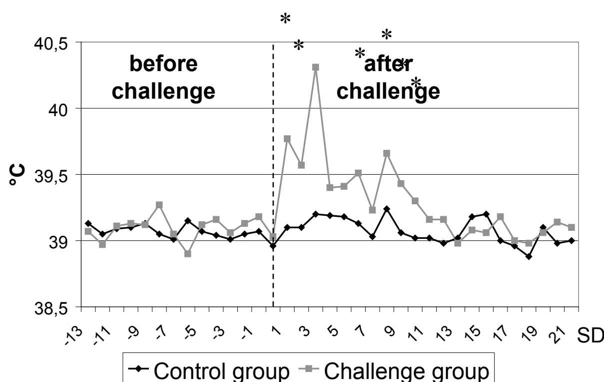
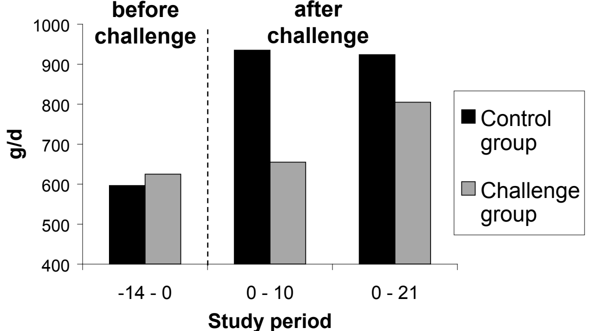


Figure 2. Average daily weight gain before and after challenge; * p = 0.011.



Discussion

The data demonstrate that a recently isolated PRRS Type 1, subtype 1 field strain is able to induce clinical signs and pathgological lung lesions in finishing pigs upon experimental challenge. As already shown by Wagner et al. 2010 (3), lung lesions were more severe ten days post infection than 21 days post infection.

Compared to the growing pigs which received a comparable challenge, the finishing pigs had a lower virus load and were also able to clear the virus faster (1) suggesting the development of potent mechanisms of innate resistance in older pigs.

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P.109

PATHOGENICITY OF VIETNAMESE HIGHLY PATHOGENIC PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS IN 2010.

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Introduction

The emerging porcine reproductive and respiratory syndrome (PRRS) outbreaks in 2006 swept over nearly half of the People's Republic of China and involved >2,000,000 pigs, which posed great concern to the global swine industry and to public health (1). A similar PRRS outbreak was also observed in Vietnam in 2007, and further spread of the disease has been found (2).

Here, we demonstrated of the disease pathogenicity using highly pathogenic PRRS virus (PRRSV) from the 2010 outbreaks in Vietnam in specific pathogen free (SPF) pigs under experimental conditions. Furthermore, we conducted a detailed investigated with real-time RT-PCR quantitative assay and pathological approach.

Materials and methods

The Vietnamese PRRSV collected in 2010 was isolated with MARC-145 cells and propagated with porcine alveolus macrophages (PAM) for experimental infection of pigs. The virus was passaged up to five times on MARC-145 cells, and two times on PAM. Thirteen 4 week-old SPF grade pigs were divided to the control (n = 4) and the challenge group (n = 9). Challenged pigs were intranasally inoculated with $10^{5.5}$ $TCID_{50}$ PRRSV/pig. Animals were monitored daily for body temperature, body weight and clinical signs. Serum was collected at 0, 6, 12, 24, 36 hour post-inoculation (hpi), 2 to 6, 8, 10, 12, 15, 17 and 19 day post-inoculation (dpi). The postmortem examination of one pig for control and 3 pigs for virus-inoculated groups was performed at 7, 14 and 21 dpi. The lungs, livers, spleens, kidneys, lymph nodes and joint liquid were collected for histopathology and the measurement of viral load. The amounts of PRRSV in the collected serum, tissues were measured by quantitative real time RT-PCR. Serum samples were tested for PRRSV antibodies using the commercial ELISA kit (IDEXX).

Results

During the experiment period, only one pig in the challenged group died at 10 dpi. All pigs in the challenged group had a high fever (40-41.7 ˚C) within 1 to 2 dpi and the fever continued until 15 to 16 dpi in the survived pigs. The pigs in the challenged group were depressed, but the red discoloration of the ear and body was not observed. In the challenged group, viral RNA in serum was detected for the first time at 12 hpi, and the peak of viral load was shown at 5 to 6 dpi. The S/P ratio of ELISA started to rise at 4 dpi and the peak was from 14 dpi. In every postmortem, the pigs had pneumonia and enlargement in various lymph nodes. The blood spots in kidneys were found in some inoculated pigs. In necropsy of the pig died, hemorrhage and consolidation of lung was observed. Histopathology showed a severe interstitial pneumonia, cell necrosis and germinal center hyperplasia in the lymph nodes, and multifocal lymphohistiocytic infiltration in many organs. The PRRSV was detected in the lungs, livers, spleens, kidneys, intestines, brains and lymph nodes of pigs in the inoculated group. The pigs in control showed no changes in body temperature and clinical sings, pathological changes and had seronegative for PRRSV antibodies throughout the experiment.

Discussion

In this present study, we'd like to attention to the measurement of viral load in the tissues and joint liquid. A large quantity of viruses was detected from lungs, kidneys, lymph nodes and tonsils by real time RT-PCR. Interestingly, the joint liquid samples were quite large amount of viral RNA ($5 \times 10^3 – 1.4 \times 10^4$ $TCID_{50}$/ml) in 14 dpi. Although the arthritis in highly pathogenic PRRS has been already reported (2), the exits of virus in the joint might play an important role for the appearance of the arthritis.

This experimental infection could be an excellent model to investigate pathogenicity of the emerging PRRSV and to evaluate vaccine efficacy for highly pathogenic PRRSV. However, the high mortality in weaning pigs reported by the Chinese PRRSV isolate was not observed in this similar experiment (3). This suggests that the high mortality of pigs in Vietnam might be caused by other pathogens with PRRSV as a major factor.

Acknowledgements

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INFECTION OF BOAR SEMEN WITH PRRSV: EFFECTS ON SPERM VIABILITY

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Introduction

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) occur worldwide and cause major economic losses in the industry today (1). The use of artificial insemination (AI) has become the standard practice, in place of natural breeding (2, 3). The objective of this study was to evaluate the parameters of infected semen with PRRS virus levels.

Materials and methods

Five mature boars of proven fertility and routinely used for AI were used. Each boar was allowed to mount a dummy and the sperm-rich fraction of the ejaculate was collected with the gloved-hand technique in a plastic bag inside. The semen was diluted to 1 x 10^6 sperm cells/ml, in 80 ml of a commercial extender (Magapor, Zaragoza, Spain), samples of 1 ml were infected with 10^2, 10^4 and 10^6 particles of PRRSV and stored for 10 days at 17°C.

To determine the quality of the spermatozoa objectively, eight parameters (Table 1), motility and progressive motility were analyzed using the CASA (Computer Assisted Semen Analyzer) at days 1, 3, 5, 7 and 10 postinfection.

Statistical analysis was done using the software SPSS 15 for Windows. Data were analyzed by means of one-way analysis of variance (ANOVA) for repeated measures.

Table 1. Definition of motility parameters.

Variable	Units	Description
Curvilinear velocity (VCL)	µm/s	Measures the sequential progression along the true trajectory
Linear velocity (VSL)	µm/s	Measures the straight trajectory of the spermatozoa per unit of time
Mean velocity (VAP)	µm/s	Measures the mean trajectory of the spermatozoa per unit of time
Linearity coefficient (LIN)	%	VSL/VCL X 100
Straightness coefficient (STR)	%	VSL/VAP X 100
Wobble coefficient (WOB)	%	VAP/VCL X 100
Mean lateral head displacement (ALH)	µm	Measures the mean head displacement along the curvilinear trajectory
Frequency of head displacement (BCF)	Hz	Measures the number of lateral oscillations of the sperm head around the mean trajectory

Results

Curvilinear velocity and mean velocity of boar semen infected with PRRSV were statistically significant ($P < 0.05$) compared to the uninfected group (Table 2).

Table 2. Motility parameters measured by CASA of boar semen infected with PRRSV (means ± S.E.M).

Days post-infection	Virus particles	VCL	VAP
1	Control	99.5 ± 4.1	56.9 ± 8.9
1	10^6	92.4 ± 3.9	52.0 ± 3.4
3	Control	88.7 ± 3.1	51.1 ± 1.6
3	10^6	96.6 ± 3.9	54.9 ± 3.5
5	Control	81.0 ± 4.0	45.7 ± 4.0
5	10^6	71.9 ± 8.1	40.1 ± 5.8
7	Control	72.8 ± 1.9	39.2 ± 1.8
7	10^6	85.3 ± 2.8[a]	50.4 ± 2.7[a]
10	Control	68.3 ± 3.1	38.5 ± 2.1
10	10^6	54.3 ± 3.4[b]	28.2 ± 2.8[b]

S.E.M.: standard error of the mean. VCL: curvilinear velocity; VAP: average path velocity. [a]$P < 0.05$ vs control group.

Discussion

In the present study, we show that boar semen infected with PRRSV induces a significant modification of different boar sperm motion parameters without any effect on motility or progressively motile spermatozoa. The UltiMate™ CASA systems can be considered as useful for *in vivo* predictive evaluation of an ejaculate.

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ENHANCEMENT OF TGF-β PROTEIN IN TONSIL AND LUNG, BUT NOT IN SERUM, OF PIGS INFECTED WITH A EUROPEAN FIELD ISOLATE OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

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Introduction

Transforming growth factor β (TGFβ) together with interleukin-10 (IL-10) are considered as immunomodulatory cytokines which are able to downregulate the host immune response, making feasible a prolonged viral replication and persistence in the organism (1,2). TGFβ is able to inhibit macrophage activation by means two mechanisms: i) inhibiting the synthesis of IFNγ (1); and (ii) promoting the production of IL-10 (3).

Several reports have suggested that IL-10 may play a significant role in the modulation of the host immune response by Porcine Reproductive and Respiratory Syndrome virus (PRRSV) (4,5). However, few reports have been focused on the expression of TGFβ during PRRS and the scarce results are controversial.

The aim of this study was to analyse the expression of TGF-β both at serum and tissue level in PRRSV-infected pigs, and to correlate with the expression of PRRSV antigen at both levels.

Materials and methods

To carry out this study, 32 pigs were inoculated with the European PRRSV field isolate 2982 and sequentially killed at 0, 3, 7 , 10, 14, 17, 21 and 24 dpi. Blood and tissue samples were collected at each time-point.

Viraemia was analysed from serum samples and the serum concentration of TGFβ was determined by a commercial ELISA kit (Biosource).

Tissue samples (lung and tonsil) were fixed in 10% neutral buffered formalin and in Bouin's solution, and processed routinely for histopathological and immunohistochemical (5). The primary antibodies used were mAb anti-PRRSV, clone SDOW-17/SR-30, diluted 1 in 1000; and polyclonal chicken anti-recombinant human TGF-β1 (R&D Systems, Minneapolis, MN), diluted 1 in 100.Differences between the means of control and inoculated animals were assessed by Mann-Whitney-U non-parametric test (GraphPad Instat 3.05). Correlation was assessed by the Spearman test (GraphPad Instat 3.05).

Results

PRRSV was first detected in blood samples at 3 dpi, showing maximum levels at 10 dpi and decreasing by the end of the study. Virus was still detected in 2/4 animals at 24 dpi. However TGFβ was not detected in sera from inoculated animals.

PRRSV antigen was detected mainly in the cytoplasm of macrophages in the lung (mainly alveolar macrophages, PAMs) and in the tonsil. Viral expression reached a maximum at 7 dpi in the lung parenchyma decreasing onwards, whereas a two-peak curve was observed in the tonsil, with a first peak at 3 dpi followed by a second peak at 14 dpi.

The pulmonary expression of TGFβ was mainly observed in PAMs and displayed a similar curve than the one observed for PRRSV ($r=0.72$; $P<0.05$). In the tonsil, TGFβ protein was chiefly observed in the cytoplasm of macrophages, displaying a maximum at 3 dpi ($P<0.05$) and decreasing onwards, but no correlation was observed with respect to the expression of PRRSV in the tonsil ($r=0.12$; $P=0.79$). The number of both PRRSV- and TGFβ-positive cells was significantly higher in tonsil than in lung ($P<0.05$)

Discussion

The lack of serum expression of TGF-β together with the local expression of this cytokine point to a paracrine effect of this cytokine in the pathogenesis of PRRS (6). In this sense, TGFβ may play a role in the immunomodulation *in situ* in PRRSV-infected tissues.

Moreover, the correlation observed in the present study between PRRSV and TGFβ antigens in lung points to a direct induction of the production of TGFβ by PRRSV, whereas the different trend observed in the tonsil suggests that an indirect mechanism might be involved in the expression of this cytokine in the tonsil.

Acknowledgements

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DIFFERENTIAL EXPRESSION OF PROINFLAMMATORY CYTOKINES IN THE LYMPHOID ORGANS OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS-INFECTED PIGS

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Introduction

Porcine Reproductive and Respiratory Sydrome (PRRS) is an economically significant disease of the modern swine industry [1]. PRRSV replication targets porcine alveolar macrophages (PAMs), macrophages in other tissues and in minor extent dendritic cells [2-3]. In this sense, viral replication has been reported in both lung and lymphoid organs of PRRSV-infected pigs [4], which suggest an important role of these organs in the pathogenesis of the disease. Moreover, PRRSV is characterised by inducing an equivocal host immune response [5] which reinforce the interest on the study of the lymphoid organs in PRRS. The aim of this study was to determine the expression of differents proinflammatory cytokines (IL-1α, IL-6 and TNF-α) in the lymphoid organs (mediastinal and retropharyngeal lymph nodes and tonsil) of PRRSV-infected pigs and to determine their correlation with the expression of PRRSV antigen.

Materials and methods

Twenty eight, five-week-old pigs were randomly distributed in batches of four, inoculated with PRRSV field isolate 2982 and killed at 3, 7, 10, 14, 17, 21 and 24 days post-inoculation (dpi). Four other pigs, were used as controls, inoculated with sterile medium and killed at the end of the study (24dpi). Samples from the mediastinal and retropharyngeal lymph nodes and tonsil were fixed in 10% buffered formaldehyde and in Bouin solution for immunohistochemical study. The primary antibodies used were mAb anti-PRRSV, clone SDOW-17/SR-30, diluted 1 in 1000; polyclonal Anti-human IL-1α diluted 1 in 100; polyclonal Anti-pig IL-6 diluted 1 in 10 and monoclonal Anti-human TNFα (clone 68B6A3) diluted 1 in 25.

Differences between the means of cell counts for control and inoculated animals were assessed by Mann-Whitney-U non-parametric test (GraphPad Instat 3.05). Correlation was assessed by the Spearman test (GraphPad Instat 3.05).

Results

In our study, PRRSV antigen was observed mainly in the medulla and/or in the paracortex of the different lymphoid tissues analyzed. The expression of PRRSV displayed a bimodal expression in all the lymphoid organs studied. The first peak of expression was observed earlier but in a milder fashion (3 dpi) in the tonsil and retropharyngeal lymph node than in the mediastinal lymph node (7 dpi).

All proinflammatory cytokines studied were mostly expressed in the lymphoreticular areas of the tonsil, mainly in the cytoplasm of macrophages and secondly in the cytoplasm of neutrophils (IL-1α) or lymphocytes (TNF-α, IL-6). Random scarce macrophages and lymphocytes were also immunolabelled against TNF-α and/or IL-6 in the lymphoid follicles of the tonsil.

In both lymph nodes all proinflammatory cytokines followed a similar trend with a statistically significant peak of expression at 3 or 7 dpi and another peak of expression at 14 dpi (IL-1α and TNF-α) or 17 dpi (IL-6). Nonetheless, whereas IL-6 was the highest cytokine expressed in the retropharyngeal lymph node, IL-1α was the one most expressed in the mediastinal lymph node, with a significant contribution of IL-1α-expressing neutrophils. On the other hand, the expression of IL-1α in the retropharyngeal lymph node was irregular showing an enhancement at the end of the study (24dpi) ($P<0.05$)

Discussion

In the present study, an early expression of proinflammatory cytokines was observed in all the lymphoid organs examined, however, their expression was different depending on each body compartment examined.

These findings may be related to a differential behaviour of PRRSV in the lymphoid organs, which may be related with the lack of a robust host immune response evoked against the virus. Therefore, lymphoid organs and proinflammatory cytokines represent an important target of study for clarifying the immunopathogenesis of PRRS.

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PHENOTYPIC CHARACTERIZATION OF IMMUNE CELLS PRODUCING IFNΓ IN PBMC AFTER IN VITRO STIMULATION WITH HOMOLOGOUS AND HETEROLOGOUS PRRSV ISOLATES

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Introduction

Both experimental and field studies underline the importance of the IFNγ-dependent immune response in the clearance of the PRRS virus. MLV seems to be effective in sustaining complete or partial protection respectively upon homologous and heterologous challenge. The degree of protection seems to be more correlated with the ability of a strain to stimulate some elements of the immune system rather than the degree of homology. On this aspect, data are still limited and not always univocal, especially with regards to the ability of triggering strong immunity regardless of genetic diversity (1, 3, 5).

The present study aims at phenotypically characterizing the fraction of immune cells producing IFNγ in PBMC after *in vitro* stimulation with homologous and heterologous PRRSV isolates in vaccinated and non-vaccinated pigs naturally infected by PRRSV.

Materials and methods

Twenty-one PRRSV naïve pigs at 4 weeks of age were randomly assigned to 3 different groups: group IM (n = 7) vaccinated with Porcilis® PRRS ($10^{4.5}$ $TCID_{50}$) intramuscularly (2 ml), group ID (n = 7) vaccinated with Porcilis® PRRS ($10^{4.5}$ $TCID_{50}$) intradermally (0.2 ml) by the I.D.A.L.® needle-less vaccinator, group ADJ (n = 7) inoculated with the adjuvant (Diluvac Forte®) only (non-vaccinated/ controls).

During the post-vaccination period (PV) the animals were kept in an isolation barn (0-35 days PV) and then moved to a conventional PRRSV-positive herd to be naturally exposed to the resident virus.

Blood samples were collected on day 0 (vaccination), 21, 35 (end of the isolation period) and on day 70 PV (35 days post-exposure). PRRSV-specific IFNγ+ immune subsets were evaluated by surface (CD8) - intracellular (IFN-γ) staining/flow cytometry in PBMC after *in vitro* re-stimulation for 48-96h with the vaccine virus (DV strain), the strain BS/114/S and the strain BS/55 (both heterologous Italian isolates) + PMA/ionomycin (last 24 h) as well as for 96h with each virus alone (2).

Results

The subsets investigated were all influenced by the onset of PRRSV infection regardless of vaccine administration: an increase of IFNγ+ cells as well as of CD8+IFNγ+ and CD8-IFNγ+ cells was detected in all groups (IM, ID, ADJ) upon *in vitro* stimulation with both the vaccine strain and the heterologous PRRSV isolates after *in vivo* PRRSV natural infection. Stimulation with the PRRS vaccine or field isolates followed by addition of PMA/ionomycin allowed to highlight slight increases of IFNγ+, $IFN\gamma+^{high}$ and CD8+IFNγ+ in the vaccinated groups at 35 days PV, especially after recall with the DV strain and the BS/55 strain. Marked stimulation of IFNγ+, $IFN\gamma+^{high}$ and CD8+IFNγ+ cells was observed after exposure to field PRRSV in both IM- and ID-vaccinated groups upon re-stimulation with the DV vaccine strain and PMA/ionomycin. Responsiveness of IFNγ+ and CD8+IFNγ+ but not of CD8-IFNγ+ subsets was detected also upon stimulation with both the heterologous strains. Lower or no stimulation was observed in the control group. Very limited stimulation and more variable results were observed upon *in vitro* recall for 96h with both the PRRSV vaccine and isolates.

Discussion

Cellular responsiveness as IFNγ+ and CD8+IFNγ+ subsets was low after PRRSV vaccination but increased after natural infection. The same results were observed by a PRRSV-specific IFNγ ELISpot assay (4). This evidence highlights that in vaccinated animals cellular immunity can be triggered upon infection although vaccination per se did not stimulate high levels of IFNγ producing cells.

In vitro re-stimulation with either the vaccine/ homologous virus and two heterologous isolates confirms that cross-reactivity against divergent viruses can be elicited and associated with CD8+IFNγ+ cells (cytotoxic cells).

These results show that the increase of IFNγ secreting cells (SC) at the ELISpot assay in vaccinated and naturally exposed pigs is associated with CD8+ cytotoxic cells and that genetic diversity between the vaccine virus and the field isolates does not seem to influence the extent of the immune cellular response (5).

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INDUCTION OF REGULATORY T CELLS DURING PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS INFECTION

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is the disease that causes the most significant losses of swine industry[1]. Recent evidence suggests that low levels of CTL and IFN-γ secreting cells PRRSV-specific could be caused by the induction of regulatory T (Treg) cells[2,3]. Two populations of Treg have been identified in swine: $CD4^{+}CD25^{+}Foxp3^{+}$ and $CD4^{+}CD8^{+}$ $CD25^{+}Foxp3^{+}CD8a^{+}MHC\text{-}II^{+}$ described as natural and activated or memory-like Treg, respectively[4]. The aim of the present study was to determinate the phenotype induced by PRRSV during acute phase of infection.

Materials and methods

Pigs were housed in the experimental farm of CIAD, A.C. The animals were infected intranasally with NVSL 97-7895 strain. Samples of serum and blood were taken at 0, 7, 14, 21 and 28 days post-infection (dpi). Viral load, anti-PRRSV antibodies, IL-10 and TGF-β cytokines and the frequency of $CD4^{+}CD25^{+}Foxp3^{+}$, $CD4^{+}CD8^{+}CD25^{+}Foxp3^{+}$, $CD8^{+}CD25^{+}Foxp3^{+}$ and $CD4^{-}CD8^{-}CD25^{+}Foxp3^{+}$ T cells were quantified. Also, the frequency Treg of cells were quantified in mediastinal lymph nodes (MLN) and tonsils from four weeks old infected (N=20) and uninfected (N=15) pigs at 0, 3, 7, 11, 18 and 24 dpi.

Results

The frequency of $CD8^{+}CD25^{+}Foxp3^{+}$ population remained stable during the time of infection. However, $CD4^{+}CD25^{+}Foxp3^{+}$ show a little increase at 28 dpi, but $CD4^{+}CD8^{+}CD25^{+}Foxp3^{+}$ cells increased two-fold at 14 dpi and three-fold at 28 dpi ($p<0.05$) (Figure 1). $CD4^{+}CD8^{+}CD25^{+}Foxp3^{+}$ Treg from MLN were detected at 3 dpi and apparently were related with the viral load in this tissue. Similarly, in tonsils $CD4^{+}CD8^{+}CD25^{+}Foxp3^{+}$ distribution increased at 18-24 dpi. The analysis of cytokine producing cells on $Foxp3^{+}$ cells stimulated in vitro with PRRSV compared with mock showed that $CD4^{+}IL\text{-}10^{+}$ cells increased 2%, $CD4^{+}CD8^{+}IL\text{-}10^{+}$ 15% and $CD8^{+}IL\text{-}10^{+}$ 10%. Moreover, in the case of $TGF\text{-}\beta^{+}$ cells PRRSV induces a slight increase on $CD4^{+}TGF\text{-}\beta^{+}$ (1.45%), $CD4^{+}CD8^{+}TGF\text{-}\beta^{+}$ (6%) and $CD8^{+}TGF\text{-}\beta^{+}$ (3%) compared to mock-stimulated cells. The analysis of cytokine mRNA expression showed an increment of IL-10 and TGF-β across the time.

Discussion

These results suggest that PRRSV induces regulatory T cells in the acute phase of infection. The phenotype induced of Treg cells are mostly $CD4^{+}CD8^{+}CD25^{+}Foxp3^{+}$. The increase in circulating Treg is a reflection of early induction on MLN at 3 dpi and is related to viral load. Also, the increase of Treg at 18 dpi in tonsil could be related to viral persistence in this tissue. In the other hand, PRRSV induce the production of IL-10 and TGF-β on $Foxp3^{+}$ cells. In conclusion, these results support our hypothesis that a delayed cellular immune response and the low production of IFN-γ by PRRSV-specific T cells can be the result of the induction of Tregs during the acute phase of PRRSV infection.

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REGULATION OF TOLL-LIKE RECEPTORS IN ALVEOLAR MACROPHAGES INFECTED BY DIFFERENT PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRAL STRAINS

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Introduction

Toll-like receptors (TLRs) are one of the most primitive pathogen-recognition systems acting in the innate immune response. Several TLRs, including TLR3, TLR7/8 and TLR9 are involved in antiviral responses by triggering the production of type I interferons (IFN). Published information about the involvement or regulation of TLRs in porcine reproductive and respiratory syndrome virus (PRRSV) is scarce and it is unclear how TLR expression is influenced or not by PRRSV replication. Moreover, considering the immunobiological diversity of PRRSV (1, 2) it would be even possible that different PRRSV strains produced different effects on TLR expression.

The aim of this work was to assess the kinetics and regulation of endocytic TLR3, TLR7 and TLR9 expression in porcine alveolar macrophages (PAMs) infected by three different PRRSV isolates.

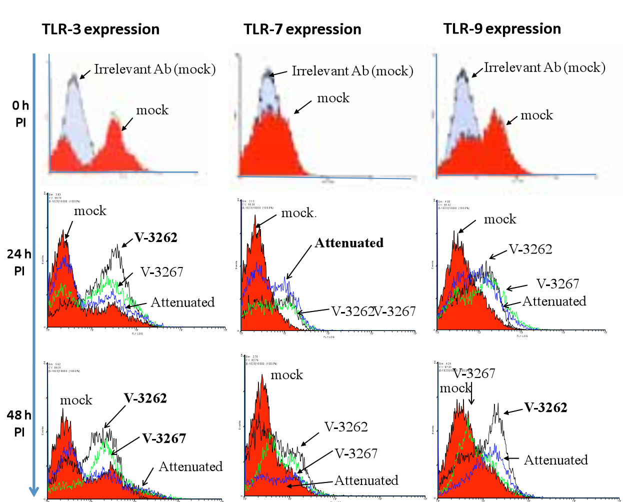
Materials and methods

Cultures of PAMs were obtained from 4 week-old pigs free of PRRSV and PCV2. Cells were infected with PRRSV isolates 3262, 3267 (1, 2), or an attenuated strain (obtained from a commercial vaccine) at multiplicity of infection of 0.1. Cells were harvested at 0h, 6h, 12h, 24h and 48h post-infection (PI), permeabilized with PBS-Tween20 (0,2%) and analyzed by flow cytometry with primary polyclonal antibodies directed to TLR3, TLR7 and TLR9 and DyLight 488-conjugated anti-rabbit IgG secondary antibody (EPICS MC2-XL). Uninfected PAMs were included at each time point as controls. The experiment was repeated twice.

Results

At time 0h PI, a high percentage of mock-infected PAMs showed a basal expression (mean±SD) of TLR3 (43±6%) and TLR9 (46±7%) that decreased over time until 48 h. This fact was observed with different batches of PAM from different animals. PAMs cultured with 3262 showed an increased expression of TLR3 at 24 h PI compared with the uninfected controls or to PAM stimulated with other strains. Also, infection with strain 3262 resulted in increased TLR9 expression at 48h (Figure 1). No evident changes were observed for PRRSV strain 3267 compared to non-infected cells except for an increase expression of TLR3 at 48h PI. For TLR7 all viral isolates produced a similar pattern of expression.

Figure 1. Histograms for flow cytometry analysis of TLR expression in macrophages infected with different PRRSV strains.



Discussion

In a previous paper (2) is shown that strain 3262 induced TNF-α release in dendritic cells. Here, that this strain induced an earliest TLR3 expression compared to a non-TNF-α inducing strain (3267). These results would support a connection between the immunophenotype of a PRRSV strain and the regulation of TLRs. Other works however, suggested that PRRSV can produce transient inhibitions of TLR3 and TLR7 (4). In conclusion, results of the present study suggest that different PRRSV strains may regulate differently the expression of endocytic TLRs. The biological meaning of this different ability in the course of infection needs further investigation.

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COMPARISON OF PRO-INFLAMMATORY CYTOKINES GENE EXPRESSION AND PROTEIN LEVELS IN TONSIL AND SERUM OF PRRSV INFECTED PIGS

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Introduction

PRRSV replicates in porcine alveolar macrophages, monocytes and dendritic cells (1). Initially these cells do not recognize the virus, occurring a down regulation of inflammatory cytokines which delay the onset of the innate immune response and further development of specific adaptative immune response (2).

The aim of this work was to determine the relationship between the mRNA expression, tissular protein expression and serum concentration of pro-inflammatory cytokines (IFN-α, IFN-γ, IL-12 p40 and TNF-α).

Materials and methods

Thirty-two PRRSV-free pigs (5 weeks old) were selected and randomly located in batches of four animals. Seven groups were inoculated by the intramuscular route with 1 ml of the third passage of PRRSV field isolate 2982 (kindly provided by Dr. E. Mateu) at $10^{3.0}$ $TCID_{50}$. Animals were euthanized at 3, 7, 10, 14, 17, 21 and 24 days post inoculation (dpi). The control group was euthanized at 24dpi.

Immunohistochemistry procedures for IFN-α, IFN-γ IL-12 p40 (3), and for TNF-α (4) were deveopled as previously related by Barranco.Pro-infamatory cytokines serum concentration was assessed by means of ELISA assay (5,6). The mRNA was extracted with the RNeasy Mini Kit (QIAGEN). cDNA was obtained by means of a retrotranscriptase-PCR, and it was used as template for q-PCR using SYBR-green chemistry (Applied Biosystems).

The standard curve was obtained integrating cDNA into a plasmid and samples were analyzed in duplicate. Gene expression was calculated as target/reference ratio of each sample normalized by target/reference ratio of endogenous calibrators (β-actine, cyclophilin and GAPDH).

Results

IL-12 p40 gene expression was elevated 182 times at 21dpi. IFN-γ was increased 2.77 times at 7 dpi and 2.51 times at 14dpi, compared to control. IFN-α and TNF-α gene expression did not change in tonsil over the experimental period.

Discussion

The fact that the pro-inflammatory cytokines gene expression change was low and delayed, confirm the immunosupressor effect of PRRSV. Our data suggest that PRRSV impaired macrophages capacity for TNF-α production, which is in agreement with the results published by López-Fuertes et al. (7).

Pro-inflammatory cytokines gene expression in tonsil did not have the same pattern than tisular protein expression and serum levels. Obtained data suggest that cytokines secretor cells located in tonsils have no important relevance in the total quantity of cytokines observed in blood. Maybe the most important effect of these cytokines was in the local area (tonsil) and not a general effect.

It would be interesting to determine the gene expression of NF-κB in futures studies. NF-κB is an inducible transcription factor involved in pathways of type I IFN, pro-inflammatory cytokines and other molecules important to stablish the immune response (8).

Quantification of cytokine gene expression in lungs and their regional lymph nodules is needed to enhance the knowledge on immunomodulatory cytokines role.

Acknowledgements

Thank Dr. E. Mateu (Universitat Autònoma de Barcelona, Spain) for providind the PRRSV field isolate 2982. This work was founded by the project No AGL2009-GAN.

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RELATIONSHIP BETWEEN IMMUNOMODULATORY CYTOKINES GENE EXPRESSION AND PROTEIN LEVEL IN TONSIL AND SERUM OF PRRSV INFECTED PIGS

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Introduction

PRRSV replicates mainly in porcine alveolar macrophages and, to lesser extent, in monocytes and dendritic cells (1). American strains of PRRSV inhibit the antigen presentation by specialized immune cells and stimulate the secretion of immunomodulatory cytokines (IL-10 and TGF-β) (2). Infection with european strains showed that dendritic cells only overexpress IL-10 whereas TGF-β synthesis was not changed (3).

IL-10 inhibits antigen presentation and dendritic cells maduration, reducing the expression of SLA-II and proinflamatory cytokines (4).

The aim of this study was to determinate the gene expression levels of IL-10 and TGF-β in tonsils and to compare them with the protein concentration in the same tissue and serum levels in experimentally infected pigs with an european strain of PRRSV.

Materials and methods

Eight randomly assigned groups (four animals per group) of 5 weeks old pigs specific PRRSV free were included in this trial. The animals were inoculated with 1 ml of $10^{3.0}$ $TCID_{50}$ of PRRSV field isolate 2982 (transferred gently by Dr. E. Mateu) intramuscularly, and the control group was mock inoculated.

Tonsils and serum samples were obtained at 3, 7, 10, 14, 17, 21 and 24 days post inoculation (dpi). The control group was obtained at 24 dpi. cDNA was obtained by means of a retrotranscriptase-PCR, and it was used as template for q-PCR using SYBR-green chemistry. The standard curve was obtained integrating cDNA into a plasmid. Samples were analyzed in duplicate. Gene expression was calculated as target/reference ratio of each sample normalized by target/reference ratio of endogenous calibrators (β-actine, cyclophilin and GAPDH). Gene expression of IL-10 and TGF-β was compared to ELISA and IHQ results. ELISA and IHQ were performed as previously reported (5, 6, 7).

Results

The gene expression did not change along the whole study. The gene expression for IL-10 in tonsils showed a peak between 17dpi (93.28 times more) and 21dpi (73.62 times more) compared with control.

Discussion

The gene expression in tonsils of TGF-β did not change as was expect in PRRSV European strain infected pigs (3). IL-10 gene transcription level and protein expression (7) in tonsils and seric levels (5) showed different profiles over the studied period.

According to other reports (3), this european strain has an unbalanced ability to stimulate T cell immune responses. The induction of swine regulatory T cell can be determinated by TGF- β, and not by IL-10.

It is possible that the effect of IL-10 is more important locally in the tonsil, rather than a general level in pigs infected with PRRSV. IL-10 can inhibit the transcription and translation of a variety of inflammatory cytokines (8).

Probably, the main production of IL-10 occurs in lungs, which is the most important tissue of PRRSV replication. IL-10 gene expression quantification in lungs from PRRSV European strains infected pigs would be interesting to enhance the knowledge on the immunomodulatory cytokines role.

Acknowledgements

We thank Dr. Enric Mateu for his kind gift of PRRSV field isolate 2982.This work was founded by the project No AGL2009-GAN.

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IMMUNOGENICITY OF AN ORALLY INOCULATED TRANSGENIC BANANA PLANT EXPRESSING THE RECOMBINANT GP5 PROTEIN OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) IN PIGS

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Introduction

Vaccines produced by transgenic plants constitute a promising alternative to conventional immunogens, presenting the possibility of stimulating humoral and cellular immunity against pathogens, invading or invading through the mucosa, when administered orally. Thus, it is feasible using transgenic plant oral vaccine to activate common mucosal immunity to prevent PRRSV infection from the first defense line. The envelop glycoprotein 5 (GP5), a key immunogenic protein of PRRSV, is the leading target for the development of the new vaccines against PRRSV (3). Our previous study has demonstrated that GP5-expressing transgenic tobacco plant could indeed induce both mucosal and systemic immunity in pigs by oral administration (2).From the purpose of application in the future, transgenic banana, especially the fruit body of banana, is more edible and easy to be accepted than the transgenic tobacco plant. The objective of the present study was to further evaluate whether similar immune responses could also be induced in pigs using the GP-5-transgenic banana plant (GP5-B) through feeding.

Materials and methods

Virus – Strain MD-001, a field isolate from a PRRSV-infected pig in Taiwan and genetically related to North American serotype isolates, was used. ***Designing of banana expression vector*** – The PCR product of PRRSV-ORF5 containing a plant-specific endoplasmic reticulum retention signal (HDEL) was inserted into an intermediate plant binary pGreen vector, which contains β-glucuronidase (GUS) gene and kanamycin resistance gene (npt II), driven by the cauliflower mosaic virus 35S promoter. The Mh-UBQ1 3' flanging region (Mh-UBQ1 3' FR) signal peptide may increase the expression level of fusion protein in transgenic plants (Fig. 1). ***Transgenic plant*** – The banana plant, *Musa* spp. 'Pei-Chiao'AAA group, was transformed through leaf discs mediated by *Agrobacterium tumefaciens* LBA4404 with the PRRSV-ORF5 expression cassette. ***Animals*** – Twelve 8-week-old, PRRSV-free, healthy pigs were used. ***Oral inoculation*** – Six pigs were fed three times with 50 g of GP5-B (GP5 reaching 0.03% of total soluble protein) each on weeks 0, 2, and 4. Six pigs were fed with 50 g of wild-type banana plant (W-B) as control group. Samples of serum, saliva, and peripheral blood mononuclear cells (PBMC) were collected at designated time points and analyzed for anti-PRRSV total IgG, neutralizing antibodies and PRRSV-specific blastogenesis, respectively.

Results

The GP5-B elicited significantly higher PRRSV-specific lymphocyte blastogenic response than that of W-T (*$P < 0.05$*), as early as 3 weeks post-initial immunization (WPI), and an immunization frequency-dependent gradual increase in the level was observed thereafter (Fig. 2). A similar gradual increase in serum anti-PRRSV IgG and saliva anti-PRRSV IgA antibody responses was also noted (Fig. 3); significant elevation appeared after the 2nd feeding of GP5-B and the levels further increased following subsequent booster. Pigs fed on GP5-B also developed serum neutralizing antibodies (NAs) to PRRSV at a titer of 1 : 4 after the 3rd feeding by 5 WPI and displayed a further enhancement in serum NAs, (1 : 8), at 7 WPI.

Fig. 1 *Plant expression vector pGKU-35PRRSV for ORF5.*

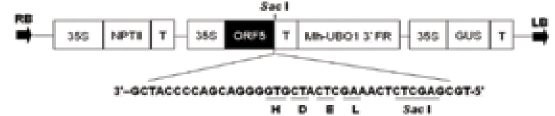


Figure 2. *Changes in PRRSV-specific blastogenic response of PBMCs with time in pigs; arrows indicating weeks (0, 2, and 4) of immunization.*

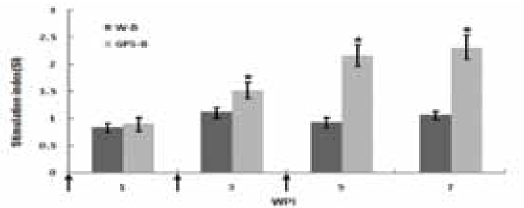
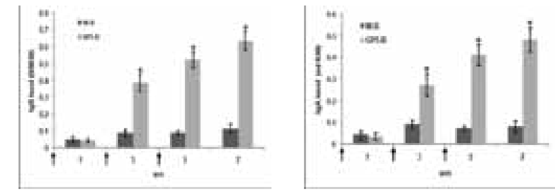


Figure. 3 *Changes in serum anti-PRRSV IgG (a) and saliva anti-PRRSV IgA (b) antibody responses with time in pigs; arrows indicating weeks (0, 2, and 4) immunization.*



Discussion

The present study has demonstrated that pigs fed orally with GP5-B could also develop specific mucosal as well as systemic humoral and cellular immune responses against PRRSV. The results support that the GP5-B can be an effective system for oral delivery of potent recombinant subunit vaccines in pigs. Although transgenic plants appear to be a promising alternative to produce recombinant antigens, the low yield of the expressed recombinant protein is still a significant and remains to be solved challenge. The low expression of GP5 in leaf tissue is speculated due to low protein content and/ or high protease activity in leaf tissue (1). In the future, the problem could be improved by feeding the pigs with fruit body of banana (higher protein ratio than the leaves) and adding the adjuvant component like *E. coil* heat liable toxin B (LTB) to the transgenic plant.

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IMPROVEMENT OF LIVE VACCINES FOR PRRS TARGETING NASAL VACCINATION PROCEDURE

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Introduction

A large number of live vaccines are used in pig farming activity. Such vaccines are based on attenuated or modified live pathogen having some infectious properties but lacking their pathogenic properties. Live vaccines can be either injected or delivered on animal mucosa and are usually not added of adjuvant. Our aim is to improve the efficacy of live vaccines by adding adjuvants in the formula or by facilitating their delivery to animals: nasal vaccines. Our findings demonstrate that in injectable PRRS model we did enhance the efficacy of a marketed live vaccine by adding an adjuvant formulation allowing to reduce the antigen content while maintaining partially the vaccination efficacy. Furthermore, we demonstrated that the same adjuvant technology could be used on pig mucosal surface with immunomodulatoring activity. We therefore plan now to work on improvement of nasal vaccination of pig.

Materials and methods

Experimental vaccines: All experimental vaccine formulations were placebo, formulated by simple dilution of the saline buffer in the adjuvants under a gentle stirring. Various Adjuvants technologies were studied: Polymeric adjuvant (Montanide™ Gel 01), Water in oil in Water (W/O/W: Montanide™ ISA 201 VG) and nanoparticles based formula (Montanide™ IMS 1313 N VG). Two trials were performed to assess the safety and efficacy of experimental vaccines.

Trial 1: All placebo formulated vaccines were administered intranasally to 4 pigs. Mucosa, musocal associated lymphatic tissues and corresponding lymph nodes were sampled and submitted to histological testing to assess the activation of immune system and/or formation of inflamation after administration of respective adjuvants.

Trial 2: Montanide™ Gel was used at 10% with 100% (4.3 log TCD_{50}/ml virus titer) or 50% of the antigenic load. A commercial not adjuvanted vaccine was tested as a positive control while a non vaccinated group was used a negative control. PRRS virus seronegative pigs weighting 10 to 15 kg were vaccinated with 2ml intramuscularly in the neck at day 0. Safety was followed by body temperature assessment after vaccination and injection site dissection at the end of the trial. Groups of 10 pigs were used. Efficacy was followed by antigen specific antibodies detection and by a challenge procedure (on day 30). After challenge the clinical signs (body temperature) presented by all animals were followed and at the end, the bacterial over-infections in lungs scored using Halbur et al., 1995 method.

Results and discussion

Trial 1: All tested formulation demonstrated an absence of general reaction: no increase of the body temperature (compared to control – untreated animals), no modification of behavior and no sign of inflamation of mucosae were detected.

On the other hand immune system was activated, because higher numbers of lymphocytes were found in respiratory tract mucosae and also in examined lymph nodes 3-7 days after administration, especially after use of Montanide™ Gel 01, Montanide™ IMS 1313 NVG and Montanide™ ISA 201 VG.

Trial 2: Formulation demonstrated an absence of general and local reaction: even when dissecting the injection site, no local reactions were found in the muscles.

The antibody titers were detected by antigen specific ELISA and no differences could be observed between adjuvanted and non adjuvanted formulation containing 100% of antigen. The formulation with adjuvant but 50% of antigen induced a reduced immune response in terms of antibody. Protection to challenge using the lung scoring was significantly superior for the adjuvanted formulation containing 100% of antigen compared to other groups. The vaccine commercially available but without adjuvant induced a similar protection as the formulation containing 50% of antigen and the adjuvant.

Conclusion

We demonstrated that adding a relevant adjuvant in live vaccine injected in pig improved the vaccine efficacy facing an infectious challenge. Furthermore, the use of such polymeric adjuvant could improve the protection conferred by a vaccination using 50% of the antigenic load to an equivalent level compared to commercial formulation containing a full antigen dose. This opens a door to modification of the vaccine formulation allowing a better control of the vaccine safety and improved efficacy. At last, the ability to use several adjuvant technologies for nasal immunization, allow thinking to needle free vaccination procedure by nasal spray.

Acknowledgements

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PHAGE DISPLAY SCREEN FOR PEPTIDES THAT BIND N PROTEIN OF PRRSV

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Introduction

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is the causative agent of PRRS, which is antigenically and genetically heterogeneous (1). ELISA test and PCR have been used extensively for diagnostic and detection of PRRSV. The N protein of PRRSV is a basic and abundant viral protein of the virion, is extensively used as an antigen in ELISA for detecting serum antibodies (2). Phage display is a powerful tool to select peptides or proteins with high affinity and specificity to almost any molecular target of interest (3). The aim of this study was select phage clones for diagnostic of PRRSV.

Materials and methods

A M13 phage random 7-mers peptide library was used to screen N protein mimotopes of PRRSV. The phage library was subjected to affinity selection as described by Ph.D.-C7C kit. The wells of an ELISA plate were coated with 150 µg/ml of SDOW-17 anti-PRRSV nucleocapsid monoclonal antibody. The titer of the eluted phage was calculated by infecting a log-phase culture of ER 2738 *E.coli*; the colonies were counted and expressed as plaque-forming units per milliliter (pfu/mL). Subsequently, the phage eluates were amplified and concentrated using polyethylene glycol precipitation by the standard procedure. The second and third panning round were similar to the first one. Phage ELISA was performed to confirm the specificity of the positive clones.

Results

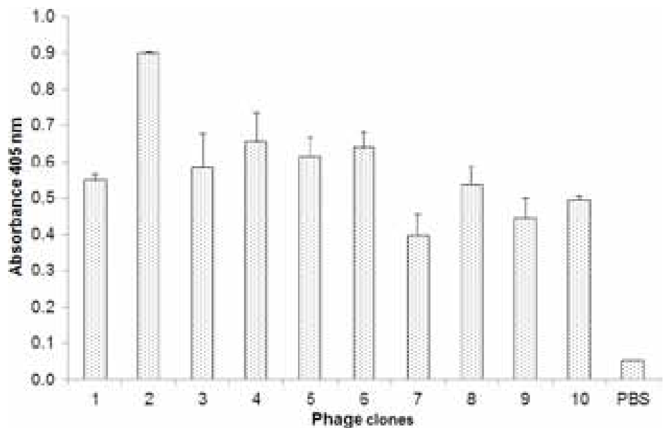
To identify peptides that can mimic structural features of N protein, we used the SDOW-17 anti-PRRSV antibodies to pan a 7-mer phage display library. In order to determine the effect of enrichment after each round of panning, input and output phages were titrated. The eluted phage of each round was increased from 3.2×10^6 pfu in the first round to 4.8×10^8 pfu in the third round (Table 1). After three rounds of panning 10 recombinant phage clones were randomly selected. The reactivity of phage clones bound to anti-PRRSV antibodies were measured by ELISA (Fig. 1).

Table 1. Enrichment of specific phages during panning.

Round	Input phage (pfu)	Eluted phages (pfu)	Phage recovery
1	1.0×10^{11}	3.2×10^6	3.2×10^5
2	1.0×10^{11}	4.1×10^7	4.1×10^4
3	1.0×10^{11}	4.4×10^8	4.4×10^3

pfu: plaque forming units

Figure 1. Sandwich ELISA to select phage clones specifically bound to SDOW-17 anti-PRRSV nucleocapsid monoclonal antibody. Arrows indicated phage clones selected. Data represent the mean of three determinations and bars represent standard deviations.



Discussion

Phage random peptide display has been employed to select mimotopes, epitope mimics that regardless of their homology to the natural antigen could serve as antigen in ELISA test. We selected two positive clones on the basis of the absorbance values above the negative controls.

Acknowledgements

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P.121
ANALYSIS OF INTRAGENIC RECOMBINATION IN ORF5 OF MEXICAN AND SPANISH ISOLATES OF PRRS VIRUS

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Introduction

Knowledge of the molecular epidemiology of a pathogen is an important element for understanding the how and why a certain variant of the pathogen gains predominance in the population at a given moment. Also, this knowledge may provide information on the forces and elements driving the genetic evolution of that pathogen. Porcine reproductive and respiratory syndrome virus (PRRSV) is thought to be one of the most variable RNA viruses (1). Most of the molecular epidemiology studies are based on establishing phylogenetic relationships based on sequencing of certain genes, particularly ORF5. However, this approach can be hampered by the existence of intragenic recombination. Recent papers (2) suggest that the generation of recombinant PRRSV viruses is probably more common than previously thought. The goal of the present study was to study intragenic recombination in ORF5 of genotype I and II PRRSV isolates.

Materials and methods

One hundred and sixty-seven ORF5 sequences from Spanish isolates (genotype I) and 148 ORF5 sequences from Mexican isolates were used. Sequences were carefully checked for quality and lack of intermediate stop codons in order to assure that they could represent the coding of true GP5 proteins. Each set of sequences was submitted to phylogenetic analysis by three different methods using MEGA 5.0: Neighbor-joining, maximum likelihood parsimony and UPGMA. In parallel, the different sets of sequences were examined for the presence of recombination single breakpoints by using the SBP utility available at www.datamonkey.org. Sequences were also examined to determine potential co-evolution of sites by using Spidermonkey/BGM. For this purpose, the 45 sites with either lower entropy values for the alignment were selected in order to found co-variation in sites with less random variation.

Results

Examination of both datasets of sequences indicated a moderate-to-high probability of intragenic recombination in ORF5 of both Spanish and Mexican sequences. Thus, in Spanish sequences a recombination breakpoint was found at nucleotide position 166 (Akaike weight support: 0.84) and in Mexican strains a recombination breakpoint was found at position 180 of the alignment (Akaike weight support 1.0). Examination of the alignments using the three different methods revealed slight differences in the alignments and clustering of the isolates. Regarding the co-variation analysis, in both cases, Mexican and Spanish isolates, clusters of co-variating nucleotides were found (n=7 and n=6, respectively). Interestingly, most of the co-variating nucleotides corresponded to the third position in the respective codon. (Table 1).

Table 1. Covariating sites in ORF5 of Spanish and Mexican isolates of PRRS virus.

	Nucleotide positions	
Group	Spanish	Mexican
1	26, 29, 138, 179, 273, 291, 298,301,345, 390, 477, 492, 553	56, 95, 162, 169, 170, 339, 429, 480, 486, 444, 495
2	224, 231, 299, 300	117, 305, 306, 411, 474, 492
3	302, 168, 558	98, 101
4	314, 414	438, 513
5	178, 327	452, 572
6	237, 525	333, 462
7	Not applicable	426, 522

Discussion

The present reports supports the notion that intragenic recombination in PRRSV occurs in field isolates and indicates that inference of evolutionary relationships of PRRSV isolates using common phylogenetic tools should be cautious if existence of recombination is not assessed. Moreover, the fact that some points of the ORF5 seem to co-evolve suggest potential functional relationship among them.

Acknowledgements

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DETECTION OF HIGH GENETIC DIVERSITY OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) IN SLOVENIA

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRS) has become one of the most economically important diseases in the pig production industry worldwide. Clinical picture is characterized by reproductive failure in sows and by a respiratory disease in young pigs. A serological survey on swine sera during 1999 - 2004 in Slovenia had demonstrated that examined herds were free of PRRS (1). As indicated by the data of a study on antibody prevalence in 194 herds, the percentage of seropositive herds increased to 44.8% in 2010 (2). PRRS is new on our territory and at the moment in the majority herds still uncontrolled swine disease. The two currently available EU genotype live-attenuated vaccines Porcilis® PRRS (Intervet) and Progressis® (Merial) are in use in Slovenia to prevent economic losses. The aim of the study was to determine the genetic diversity of PRRSV in detected positive herds.

Materials and methods

Samples

In total, 60 PRRSV positive samples were selected between 2009 and 2010 from 48 herds to determine genetic diversity of circulating field viruses. Samples originated from herds which had history of high mortality and respiratory symptoms or reproductive disorders.

Methods

Total RNA was extracted from 60 tissue suspensions using QIAamp viral RNA mini kit (Oiagen, Germany) according to the manufacturer's instructions. Samples were tested with One-Step RT-PCR kit (Qiagen, Germany) reagent using PRRSV specific primers based on the ORF 7, which detects both American and European PRRSV isolates (3). RT-PCR products were sequenced directly in both directions using the Macrogen sequencing service (South Korea) and the PCR amplification primers to confirm the specificity of the RT-PCR assays. For each sample, 258 nucleotide long sequences were aligned with the published data using BLAST (available at http://www.ncbi.nlm.-nih.gov/) at the National Centre for Biotechnology Information (NCBI). Multiple sequence alignment was carried out using the sequence analysis software Lasergene® (DNASTAR Inc., Madison, WI, USA). Sequences were aligned to the corresponding fragment of the published sequences of the EU-1, EU-2, EU-3 and EU-4 subtypes (4).

Results

Molecular epidemiology study was performed on 60 PRRSV collected in 48 positive herds and was based on 258 nucleotides of ORF 7 (genome position 14.673 - 14.927 in the Lelystad virus, GenBank acc. no. M96262). Genetic diversity of detected PRRSV revealed the circulation of least nine distantly related PRRSV strains circulating in positive pig farms in Slovenia with an 85.3 – 100% nucleotide identity to each other.

All sequenced PRRSV from 48 herds belong to subtype EU-1. In 33 (68.7%) PRRSV positive herds, almost genetically identical PRRSV strains (96.9 - 100% nucleotide identity) were identified (representative strain SI-Stra8t/2010, GenBank acc. no. HQ213911) confirming the intensive transmission of PRRSV between these herds in region with dense pig population, but the detection of PRRSV from this cluster revealed only 92% nucleotide identity with the closest sequence in GenBank. When more than one samples were sequenced in the same herd, the detected heterogeneity revealed identical sequence or only few nucleotide changes, some of them led to amino acids substitutions. In one herd new introduction of PRRSV (89.9% nucleotide identity) was confirmed after first elimination of virus. The detected nine genetic groups of PRRSV shared 89.1 - 96.1% nucleotide identity with the Lelystad virus, Porcilis® PRRS and Progressis® vaccine strains (all the EU genotype 1 of PRRSV), and a 62.4 - 65.1% nucleotide identity with VR-2332 (the NA genotype 2 of PRRSV).

Discussion

Molecular epidemiology data of the detected field strains provide first insight into the genetic heterogeneity of circulating PRRSV in Slovenia. The emergence of high number of genetic variant of PRRSV could be the result of importing live infected pigs and semen from many unknown locations followed by fast local spread of some strains of PRRSV.

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P.123
GENETIC DIVERSITY OF PRRSV STRAINS CIRCULATING IN LITHUANIAN WILD BOARS (SUS SCROFA) POPULATION

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Introduction

PRRSV strains are divided into two genotypes based on genetic and antigenic characteristics: genotype 1 (formerly European) genotype 2 (formerly North American) (3). Within the genotype 1, several genetic subtypes were defined (3, 4). A lot was reported on PRRSV prevailing in domestic swine, but still a little is known about this infection in European wild boar. Comprehensive information on PRRSV infection in wild boars was only reported from Germany (2). Wild boars have been also found seropositive for PRRSV in France, USA, Italy, and Croatia. It is not known yet whether they may act as the natural virus reservoir or a vector.

The objective of this study was to analyse the prevalence of PRRSV infections in wild boars in Lithuania and to characterize genetically the detected strains.

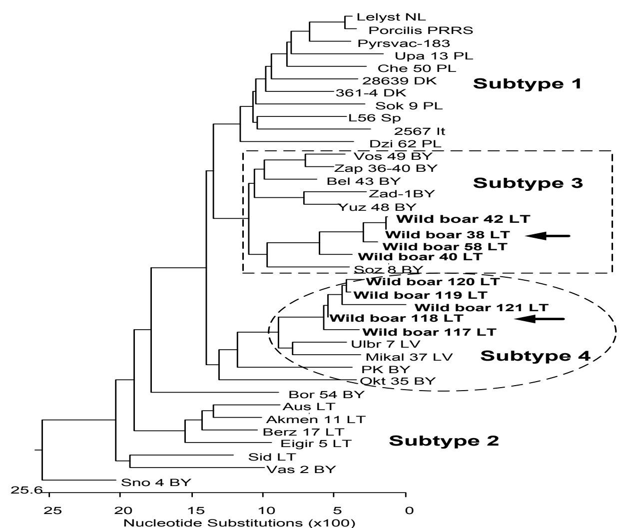
Materials and methods

Samples of blood sera and lungs of 159 wild boars were collected from 15 hunting grounds situated in 5 regions of Lithuania during autumn-winter hunting seasons from 2007 to 2011. Total RNA was extracted from homogenate of tissue or serum samples using the GeneJET RNA Purification kit (Fermentas). It was used as a template in reverse transcription nested PCR, specific for ORF5 of 1st and 2nd genotypes of PRRSV as described previously (4, 5). Gel-purified 606 bp ORF5 PCR products were cycle sequenced using the BigDye Terminator Cycle Sequencing kit (v2.0, Applied Biosystems) and ABI310 genetic analyzer. Sequence alignment was performed using the Clustal W software. A Phylogenetic tree was constructed with MegAligh program from Lasergene program package. A set of sequences representing the full genetic diversity of genotype 1 PRRSV was used as a reference.

Results

Samples of 13 wild boars (8.2%) tested PRRSV-positive in genotype 1 specific nPCR. No positive results were obtained in genotype 2 specific nPCR. Sequencing and genetic comparison of the selected amplicons revealed that these wild boar sequences belong to two genetic subtypes 3 and 4 (Fig. 1). The new sequences formed well defined clusters within these subtypes. Interestingly such strains were never found in domestic pigs in Lithuania. Subtype 3 viruses are common in Belarus and subtype 4 was found in two Belarusian and two Latvian farms.

Figure 1. Phylogenetic tree based on ORF5 nucleotide sequences.



Discussion

This study has for the first time demonstrated presence of PRRSV in Eastern European wild boars. It has shown that wild boar population can harbour different genetic lineages of PRRSV strains than those found in domestic pigs in Lithuania. This poses a serious threat for Lithuanian farms where only subtype 2 strains are circulating. Recent studies (1) showed that subtype 3 strains may be highly virulent. The most striking finding is the detection of subtype 4 strains in 5 wild boars. Previously this subtype was only detected in 4 farms in Belarus and Latvia. Altogether, these findings are strongly supporting the role of wild boars as a natural reservoir for PRRSV.

Acknowledgements

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PREVALENCE OF ANTIBODIES TO PRRSV IN WILD BOARS FROM LITHUANIA

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Introduction

PRRSV is endemic in most swine-producing countries, and is associated with major economical losses. Antibodies to PRRSV can be found in pig samples world-wide, however, positive wild boar samples were only occasionally found in some countries harboring very dense swine and wild boar populations. Most likely wild boars there became infected by domestic swine as a result of seldom direct or indirect contacts. Majority of pig farms in Lithuania were positive for PRRSV, but recently some had depopulated and became PRRSV free. Since domestic pigs and wild boars have the same susceptibility to various infections there was major concern to monitor the epidemiological PRRSV situation in feral pigs. The objective of the present study was to investigate prevalence and distribution of PRRSV antibodies in Lithuanian wild boars.

Materials and methods

A total of 659 serum samples from wild boars from 42 locations throughout Lithuania were collected during autumn–winter hunting seasons 2008/2009 and 2009/20010. The wild boars sera were analyzed via different ELISA test systems, IDEXX PRRS 2XR Ab, IDEXX HERDCHEK PRRS X3 antibody test kits (Corporate Headquarters IDEXX Laboratories, Inc., USA), Ingezim PRRS Europe (Ingenasa, Madrid, Spain) according to manufacturer's instructions and ISO/IEC 17025:2005 standard accredited laboratory.

Results

From 659 examined wild boar sera, 43 (6.5 %) were positive to PRRSV antibodies. Investigation of PRRSV antibodies with different ELISA kits did not show difference in detection positive serum samples ($p>0.05$). The results of serological analysis are summarized in Table 1. Antibodies to PRRSV were detected in all age groups; however seroprevalence was significantly higher in adult animals (Table 2). Wild boars serum samples from 31 locations out of 42 investigated were seropositive for PRRSV.

Table 1. The results of detection PRRSV antibodies in wild boars samples

Year	Number of investigated serum samples	Number of positive serum samples	% positive
2008	286	26	9.1
2009	274	15	5.5
2010	99	2	2.02
Total	**659**	**43**	**6.5**

Table 2. Prevalence of PRRSV antibodies in wild boars serum by age groups.

Age group	Number investigated	Number positive	% positive
Juveniles (up to 12 month)	227	8	3,5
Subadults (up to 24 month)	266	9	3,4
Adults (over 24 month)	166	26	15,7

Discussion

In spite of the fact that PRRSV is actively circulating in domestic swine of Lithuania, the seroprevalence in wild boars was only 6.5 %. This result indicates very low possibility of contacts between wild boars and domestic swine, which could present opportunity for PRRSV transmission. Similar results of PRRSV seroprevalence (8.92%) in feral pigs were reported in Croatia (3). However in reports from Italy the prevalence (37.7%) of PRRSV antibodies was quite high and it could be due to PRRSV transmission from domestic pigs to wild boars (2).

This is the firs report of serological evidence of PRRSV infection in the wild boar population in Eastern Europe. Interestingly, PRRSV antibodies in feral pigs were so far not detected in the neighboring countries such as Russia (1) or Poland.

Acknowledgements

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EVOLUTION OF A STRAIN OF PRRS IN A PRODUCTION PYRAMID OF PIGLETS: SEQUENCING AND DRIFT

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Introduction

One of the principal current diseases in porcine production is the Porcine Respiratory and Reproductive Syndrome (PRRS) (1). There are many techniques for following the evolution of PRRS on a production farm, each with its own value and use.

This paper discusses sequencing as a means of analysing the evolution of a strain over time. Sequencing reveals the variability in the nucleotides of the genome. The study identified the strains present in a production pyramid, and their evolution over a period of five years.

Materials and methods

The system under study was isolated from other porcine production centres, and had been negative for PRRS up to 2005. The pyramid of 14,000 females was composed of four production units: a breeding farm on a closed semi-cycle (GM); two farms for the production of 6kg suckling pigs separated by 500m (GL1 and GL2); and a farm for the production of 18kg piglets (GL3) located 6km away. Since 2005, various PRRS outbreaks have occurred, with differing degrees of pathology.

Fifteen sequencing analyses were performed on each outbreak between August 2005 and August 2010. The blood samples were sent to the Laboratori Veterinari de Diagnosi de Malalties Infeccioses (UAB) where they were subjected to a technique involving inverse transcription of the RNA to cDNA and subsequent amplification (RT-PCR ORF 5 European strains).

Using this technique, the sequences of the nucleotides of the genome of the strains from different outbreaks were compared, and the genomic variability was obtained both between strains and with reference to the Lelystad virus (M96262). It should be noted that the resultant matrix does not reflect the similarity between the strains, but rather the distance between them (2).

Results

The evolution of the initial strain (CReSA30) remained stable until October 2007. The subsequent sequencings showed a variance of 3% in relation to the initial strain, which is on the borderline of genetic drift.

On farm GL3, the sequences of the virus took a different path to the rest, as a result of the distance between this site and the others, and the continuous activity of the virus in transition. After July 2009, the strain 79-09-11R appears, with a 3.7% difference to the CReSA30 strain which increases to 4.8% in August 2010 (136-10-9R).

Table 1. Matrix of percentage of distance between strains.

		1	1	1	M	1	2	1i	2	3	3	M	1	1i	3
	Lelystad	aug-05	jan-07	oct-07	jun-08	oct-08	nov-08	mar-08	nov-08	jul-09	feb-10	may-10	apr-10	apr-10	aug-10
	M96262	CReSA30	28-07-2-F	152-6-07-F	97-08-R	116-08-3-F	128-08-3R	130-08-1-R	135-08-4R	79-09-11R	12-10-8F	69-10-4F	84-10-1F	84-10-2F	136-10-9R
M96262	0.0	6.7	6.9	8.4	7.7	7.8	7.8	7.6	9.2	8.2	9.3	7.8	7.4	7.8	8.5
CReSA30	6.7	0.0	0.8	3.4	2.5	2.4	2.9	2.2	4.1	3.7	4.8	3.2	2.4	2.4	4.8
28-07-2-F	6.9	0.8	0.0	3.6	2.7	2.5	3.0	2.4	4.1	3.9	4.9	3.4	2.5	2.5	4.9
152-6-07-F	8.4	3.4	3.6	0.0	3.6	5.1	5.7	4.9	6.8	5.8	6.9	5.6	5.1	5.1	6.9
97-08-R	7.7	2.5	2.7	3.6	0.0	4.3	4.6	3.7	5.9	5.0	6.0	4.6	4.3	4.3	6.0
116-08-3-F	7.8	2.4	2.5	5.1	4.3	0.0	0.7	0.5	2.0	5.1	6.2	1.2	0.3	0.0	6.2
128-08-3R	7.8	2.9	3.0	5.7	4.6	0.7	0.0	1.0	1.5	5.5	6.2	0.7	0.5	0.8	6.2
130-08-1-R	7.6	2.2	2.4	4.9	3.7	0.5	1.0	0.0	2.2	4.9	6.0	1.3	0.5	0.5	6.0
135-08-4R	9.2	4.1	4.1	6.8	5.9	2.0	1.5	2.2	0.0	6.6	7.3	2.0	1.7	2.0	7.5
79-09-11R	8.2	3.7	3.9	5.8	5.0	5.1	5.5	4.9	6.6	0.0	2.0	5.8	5.1	5.1	1.7
12-10-8F	9.3	4.8	4.9	6.9	6.0	6.2	6.2	6.0	7.3	2.0	0.0	6.2	6.2	6.2	1.7
69-10-4F	7.8	3.2	3.4	5.6	4.6	1.2	0.7	1.3	2.0	5.8	6.2	0.0	0.8	1.2	6.2
84-10-1F	7.4	2.4	2.5	5.1	4.3	0.3	0.5	0.5	1.7	5.1	6.2	0.8	0.0	0.3	6.2
84-10-2F	7.8	2.4	2.5	5.1	4.3	0.0	0.8	0.5	2.0	5.1	6.2	1.2	0.3	0.0	6.2
136-10-9R	8.5	4.8	4.9	6.9	6.0	6.2	6.2	6.0	7.5	1.7	1.7	6.2	6.2	6.2	0.0

Discussion

Following the strains running through each of the processes leads to information on the presence or otherwise of any new strains. Thus adequate biosecurity measures can be put in place, and the response to change in the adaptation to the replacements can be evaluated.

With the sequences obtained in this study, it was not possible to identify any new strains entering the pyramid. The changes in the homology were caused by random drift, owing to the recirculation of the virus in the susceptible population. With respect to the strains on farm GL3, the effect was accentuated as a result of the activity of the virus in transitional piglets.

Because of the mutation of the virus, the immunisation program for the replacement pigs needed to be modified, while also adapting and continuing with the existing vaccination schedule in order to avoid instability in the investigation.

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PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) SEROPREVALENCE IN VENEZUELA

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Introduction

Porcine Reproductive and Respiratory Syndrome Virus has been reported as a cause of high mortality in farms around the world. In Venezuela, the first evidence on the presence of PRRSV was found in 1998 by seroprevalence studies, where a wide distribution of infection was found in the states where most of the porcine industry was concentrated by that time (1). The aim of this study was to evaluate the seroprevalence for PRRSV during the years 2008, 2009 and 2010.

Materials and methods

A total of 6.306 sera samples from sows and postweaning pigs between 4-10 weeks of age from farms located in 15 states from Venezuela were evaluated between January 2008 and December 2010 by "Empresa de Diagnóstico Veterinario C.A.". The blood samples were taken by jugular venipuncture and centrifuged at 1.500 rpm during 7 minutes and the serum was stored in tubes at -20 C.
These samples were used to detect antibodies against Porcine Respiratory and Reproductive Syndrome virus (PRRSV) through an ELISA test (HerdCheck* PRRS Virus Antibody Test Kit 2XR, IDEXX Laboratories). All the samples were processed according to the protocol described in the ELISA kit (IDEXX Laboratories) and read using a spectrophotometer at 450 nm. Results were expressed on S/P values, where all sample with S/P>=0.4 were considered as positive.

Results

The percentages of positive samples during 2008, 2009 and 2010 were 14,33%, 12,01% and 16,44%, respectively. In 2008, the states of Portuguesa, Cojedes and Aragua, showed the largest percentage of positive samples; In 2009 Carabobo, Yaracuy and Aragua and finally in 2010, Carabobo, Cojedes and Aragua were the sates with the highest seropositivity (Table1).

Discussion

In Venezuela, the highest proportion of the porcine population is located in the central regions, including the states of Aragua and Carabobo, where farms are managed under a continuous flow system without biosecurity measures, which likely explains the higher seropositivity found in the animals tested in these states. The results are shown on table 1.

On the other hand, a considerable decrease in the PRRSV seropositivity was observed compared to previous reports (1,2); this was probably due to the implementation of management measures aimed at PRSS control.

Because PRRS vaccination was not allowed in Venezuela at the moment of this study, we conclude that the seroprevalence observed was associated with viral infection in the farms tested.

Table 1. Percentage of positive serum samples to antibodies against PRRSV in 15 farms from Venezuela between January 2008 and December 2010.

	2008			2009			2010		
State	**N°**	**+**	**%**	**N°**	**+**	**%**	**N°**	**+**	**%**
Anzoát.	0	0	0	0	0	0	21	0	0
Aragua	386	108	**28**	128	22	**17**	489	72	**15**
Barinas	181	30	17	0	0	0	0	0	0
Carabob.	781	206	26	377	134	**36**	763	351	**46**
Cojedes	74	23	**31**	0	0	0	76	29	**38**
Falcón	36	2	6	15	0	0	15	0	0
Guarico	1066	31	3	580	14	2	777	0	0
Lara	406	0	0	410	10	2	495	8	2
Mérida	50	0	0	33	5	15	0	0	0
Miranda	267	2	1	142	2	1	0	0	0
Portug.	98	38	**39**	0	0	0	128	0	0
Táchira	0	0	0	0	0	0	7	0	0
Trujillo	0	0	0	0	0	0	0	0	0
Yaracuy	613	124	20	107	35	**33**	0	0	0
Zulia	40	0	0	56	0	0	27	0	0

Total	N° Samples	Positive	Percentage
2008	3.998	573	14,33%
2009	1.848	222	12,01%
2010	2.798	460	16,44%

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FIRST OUTBREAK OF HP-PRRSV, A CHINESE-LIKE STRAIN IN THAILAND

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is a swine virus causing major economic losses in the swine industry worldwide. PRRSV is divided into 2 genotypes, European (Type 1) and North American (Type 2) genotypes. PRRSV have highly genetic variation particularly at the non-structural protein 2 (nsp 2) and ORF 5 (Ref Kedkovid &?). In 2006, a highly pathogenic PRRSV (HP-PRRSV) or swine high fever syndrome virus was found in China (1). Whole-genome analysis of the isolated virus was identified and grouped in PRRSV type 2. Interestingly, HP-PRRSVs contain a deletion of 30 amino acids in nsp 2. In Thailand, HP PRRSV has not been reported before 2010 (2). In July 2010, backyard pigs of all age groups in Nongkai province (north-east) showed HP-PRRSV-like symptom such as depression, high fever, respiratory signs and hemorrhages at all organs with high mortality rate. A few months later, the Chinese-like PRRSV emerge in Phitsanulok province (central part of Thailand) with similar symtomp found in Nongkai province. This is the first report of HP-PRRSV Chinese-like strain outbreak in Thailand.

Materials and methods

Serum and lung tissues were collected from dead and sick pigs from both provinces. The PRRSV RNA was extracted and used for RT-PCR. We used primers specific to nsp2 of PRRSV (Forward: 5′-AAA GAC CAG ATG GAG GAG GA-3′ and Reverse: 5′-GAG CTG AGT ATT TTG GGC GTG-3′) (3). The purified PCR products were sequenced. The nucleotide sequences were aligned in MEGA 5. A phylogeny was constructed using neighbor-joining method with 1000 bootstraps on the alignments with the program MEGA 5.

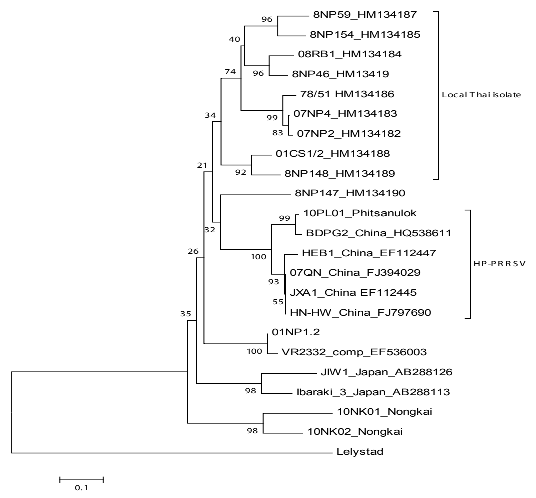
Results

PRRSV from Nongkai province (10NK01, 10NK02) had amino acid deletion at position 936-943 of nsp2. PRRSV from Phitsanulok (10PL01) contained 30 amino acids deletion at position 864 and position 920-948 of nsp2. The phylogenetic analysis showed closely related between 10PL01 and HP-PRRSV from China (BDPG 2)(figure 1).

Discussion

The first outbreak at the Lao PDR borderline (Nongkai province) showed similar Chinese HP-PRRSV-like clinical signs to the outbreak in Phitsanulok province. However, genetic analysis of the Nongkai viruses (10NK01, 10NK02) was in the different clade from 10PL01 and HP-PRRSV from China. Although the deletion in the nsp2-coding region was not related to the virulence of the emerging PRRSV in China, it could be used to identify the origin of virus since similar clinical manifestation may not always cause by the same virus. It is possible that these two outbreaks occurring in Thailand caused by different PRRSV sources. Due to unavailable genetic data from the neighboring countries the investigation of the origin of Nongkai viruses (10NK01, 10NK02) is limited. Illegal smuggling of vaccines and animal movement between neighborring countries are major problems of introducing new pathogens into the country.

Figure1. Phylogenetic tree of nsp2 amino acid sequence



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LONGITUDINAL STUDY OF PORCINE REPRODUCTIVE AND RESPIRATORY DISEASE VIRUS IN TWO ENDEMIC FARROW-TO-FINISH FARMS

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Introduction

It is a common assumption that porcine reproductive and respiratory syndrome virus (PRRSV) spreads easily among weaners and fatteners. Moreover, in endemic farms, seroprevalence of PRRS is close to 100% in finishers. Under natural conditions, the infection is assumed to be transmitted horizontally through oral or nasal fluids, either in aerosols or trough injured/abraded skin (1,2). Nonetheless, few studies have been done to assess the dynamics of PRRSV infection in weaners and fatteners under field conditions. The objective of the present work was to study the dynamics of PRRSV in endemic farms.

Material and methods

Two seropositive to PRRSV farrow-to-finish farms (F1 and F2) were selected for this study. In each farm a batch of pigs (n=79 and n=115, respectively) was followed from weaning to slaughtering age. Blood samples were obtained weekly from the 3 to the 21 week of age in F1. In F2, blood sampling was done weekly from 3 to 13 weeks of age and then animals were bled at 15, 17 and 24 weeks of age. In each visit to the farms clinical observation and pen allocation of each individual pig at each given time was recorded. A commercial ELISA (Ingezim PRRS, Ingenasa) was used for the detection of PRRSV antibodies.

Results

In F1, seroconversions started between 12 and 15 weeks of age. In F2, seroconversions started after the decay of maternal antibodies in between 3-6 weeks of age. Both farms presented some negative animals at the end of the study. The tables 1 and 2 show the incidence for each three-week period and the cumulative incidence for both farms. Figure 1 shows the temporal and spatial distribution of cases.

Table 1. Incidences of PRRSV infection in Farm 1.

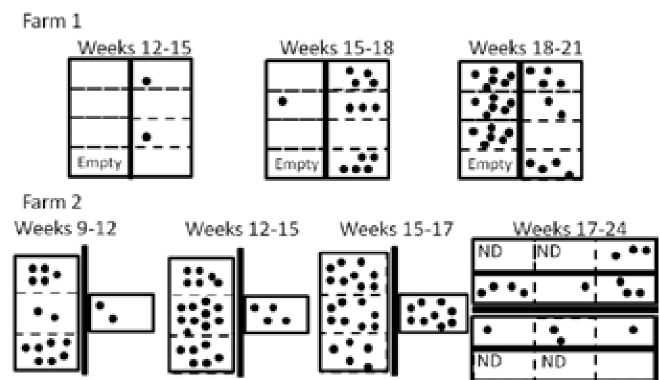
Farm 1		
Weeks	3-week Incidence	Cumulative incidence (%)
3-6	0	0
6-9	0	0
9-12	0	0
12-15	2.6	2.6
15-18	17.6	19.7
18-21	54.1	63.2

Table 2. Incidences of PRRSV infection in Farm 2.

Farm 2		
Weeks	3-week Incidence	Cumulative Incidence (%)
3-6	4.3	4.3
6-9	0	4.3
9-12	15.7	19.6
12-15	31.7	45.2
15-17	51.9	73.5
17-24*	68.2	93.3

* Incidences were calculated for a 7- week period.

Figure 1. Temporal and spatial distribution of seroconversions in farms 1 and 2



Seroconversion; – – Discontinous separation; ND=not done

Discussion

Results obtained in this study show that a relatively long period of time (9-12 weeks) was needed for the virus to spread to the majority of pigs although seronegative pigs remained at the end of the fattening period. The situation observed would be compatible with a low infection transmission and where close and repeated contact between animals would be needed to produce effective transmission.

Acknowledgements

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ALIGNMENT OF FREQUENTLY USED PRIMER PAIRS TO DETECT THE ORF5 REGION FROM EUROPEAN PRRSV FIELD STRAINS

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Introduction

EU-genotype PRRS viruses were assumed to form the homogenous “Lelystad-like” group, which has already been disproved. In the present study, the theoretical possibility of the detection of PRRSV sequences from different European countries by already published primer pairs was tested. The aim was to find out, if field strains are already too heterologous for detection by a randomly selected method and if the risk of false-negative results is increasing.

Materials and methods

A total of 100 PRRSV genome sequences of the ORF5 region from different European countries were retrieved from GenBank and aligned against the Lelystad strain M96262 by ncbi/BLAST/blastn (Tab. 1). The sequences were aligned to several primers already published for this genomic region (Tab. 2).

Results

The various PRRSV strains showed 85 to 99 % homology with the Lelystad strain. Primers established by different authors were similar and placed in comparable genomic regions. Fig. 1 shows the alignment of the end of ORF4, ORF5 and start of ORF6 of the Lelystad strain (13201-14220bp) and several primers. Divergent primers are marked grey, start and end of ORF5 by arrows. 5 of 22 primers were not 100 % matching with M96262. The heterogeneity within the ORF5 region of EU PRRSV strains had no impact on the selection of primers for PCR analysis as long as the primers corresponded to the M96262.

Fig. 1: Alignment of Lelystad strain and primers
(I = inosine = complement to C or A, Y = pyrididine = C or T)

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  13201 ACGGCTAACG  TGACCGACGA  ATCATACTTG  TACAACGGGG  ACCTGCTGAT  GCTTTCTGGG
    9fw                                                        AT  GCTTTCTGGG
  13261 TGCCTTTTCT  ACGGCTCAGA  AATGAGGGAG  AAAGGCTTCA  AAGTCATCTT  TGGGAATGTC
    9fw TGCCTTTTC
  13321 TCTGGGGTTG  TTTCTGCTTG  TGTCAATTTC  ACAGATTATG  TGGCCCATGT  GACCCAACAT
  13381 ACCCAGCAGC  ATCATCTGGT  AATTGATCAC  ATTCGGTTGC  TGCATTTCCT  GACACCATCT
    3fw                                      CGGTTGC  TIGATTTCIT  GAC
    9fw                                      CGGTTGC  TGCATTTCCT  GAC
    9fw                                         TTGC  TGCATTTCCT  GACACC
  13441 GCAATGAGGT  GGGCTACAAC  CATTGCTTGT  TTGTTCGCCA  TTCTCTTGGC  AATATGAGAT
1,4,5fw  CAATGAGGT  GGGCIACAAC  C
  2,8fw     TGAGGT  GGGCTACAAC  CATT
    6fw  CAATGAGGT  GGGCTACAAC  C
    7fw                                                             ATATGAGAT
    9fw    ATGAGGT  GGGCTACAAC  CATTGC
  13501 GTTCTCACAA  ATTGGGGCGT  TTCTTGACTC  CGCACTCTTG  CTTCTGGTGG  CTTTTTTTGC
    7fw GTTCTCACAA
          Between 13560 and 14040 no primers were aligned.
  14041 TCGAAGGGGT  TAAAGCTCAA  CCCTTGACGA  GGACTTCGGC  TGAGCAATGG  GAGGCCTAGA
    7re                                     ACTTCGGC  TGAGCAATGG  GAGGCCTAG
  14101 CGATTTTTGC  AACGATCCTA  TCGCCGCACA  AAAGCTCGTG  CTAGCCTTTA  GCATCACATA
1,4,5re                                          GTG  CTAGCCTTTA  GCATIACATA
  2,8re                              ACA  AAAGCTCGTG  CTAGCCT
    6re                                          GTG  CTAGCCTTTA  GCATCACATA
    9re         GC  AACGATCCTA  TCGCCGCACA
  14161 CACACCTATA  ATGATATACG  CCCTTAAGGT  GTCACGCGGC  CGACTCCTGG  GGCTGTTGCA
    3re              TGATATAIG  CCCTIAAGGT  G
    9re             ATGATATAYG  CCCTTAAGGT
```

Table 1. Accession numbers, origin and maximum identity with the Lelystad strain M96262

Acc. no.	Origin	Maximum identity (%)			
		≤ 90	91-95	96-98	99
AY615786 - AY615796; AY875853 - AY875862	Austria n=21	5	13	3	0
H-01-1d,e,f; H-03-1a,b; H-05-1a; H-06-1a; H-10-1c; H-16-1a; H-20-1a,2a; H-26-2a,2c,2d; H-29-1b; H-30-1b,2b; H-45-1a; H-46-1a; H-47-1a; AF378797; AF378798; AY035912; AY035922 - AY035925	Ger-many n=27	16	3	6	2
A F 3 7 8 7 9 9 ; AY035938 - AY035940	Great Britain n=4	0	0	3	1
AF378804 - AF378818	Poland n=15	6	9	0	0
AY035900 - AY035901	Belgium n=2	0	0	2	0
AF253531	Czech n=1	1	0	0	0
AY035902 - AY035917; AY035944	Den-mark n=15	9	6	0	0
AY035919 - AY035920	France n=2	0	0	2	0
AY035926 - AY035933; AY035941 - AY035943	Italy n=9	6	1	2	0
L04493	Nether-lands n=1	0	0	0	1
AY035935 - AY035937; DQ345755	Spain n=4	2	2	0	0

Table 2. List of primers for the amplification of ORF5 from EU-PRRSV strains.

No.	Reference
1-3	Greiser-Wilke et al. (2009): Vet. Microbiol. 143, 213-223.
4	Oleksiewicz et al. (1998): Vet. Microbiol. 64, 7-22.
5	Forsberg et al. (2002): Virology 299, 38-47.
6	Indik et al., (2000): J. Gen. Virol. 81, 2497-2502.
7	Stadejek et al. (2002): J. Gen. Virol. 83, 1861-1873. Nested PCR: inner primers (outer primers: ad No. 4)
8	Mateu et al. (2003): J. Gen. Virol. 84, 529-534.
9	Pesch et al. (2005): Vet. Microbiol. 107, 31-48.

Discussion

In the present study, the comparison was carried out over theoretical alignments and not controlled by practical PCR methods. As the chosen PRRSV strains had been detected during the last 15 years, it cannot be concluded that newly established strains can be detected with the same accuracy. The necessity of evaluating the current epidemiological situation is still given.

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RELIABLE DETECTION AND DIFFERENTIATION OF PRRSV FIELD ISOLATES AND THE EUROPEAN VACCINE STRAIN USING A VACCINE-SPECIFIC RT-PCR (DV-PCR)

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is the causative agent of one of the most economically important pig diseases to be found anywhere in the world. Germany has a leading role in the European pig production industry. For the German pig producing industry, it is of the utmost importance to know as much as possible about the circulation of PRRSV within its pig-dense regions, and crucial to be able to differentiate between European field strains and the avirulent vaccine strain of Porcilis® PRRS (Intervet/ Schering-Plough).

Several veterinary diagnostic laboratories offer a so-called DV-PCR (Harder et al., 2004) which is specific for the ORF5 gene of Porcilis® PRRS.

The aim of the present study was to analyze the sensitivity and specificity of this PCR in order to be able to guarantee reliable results for both veterinarians and farmers.

Materials and methods

A total of 78 European field isolates of PRRSV from the routine diagnostic facility of the institute of virology were analyzed in the DV-specific ORF5 RT-PCR (1). Complementary DNA was synthesized after random-primed reverse transcription, using the Superscript II polymerase (Invitrogen, Germany).

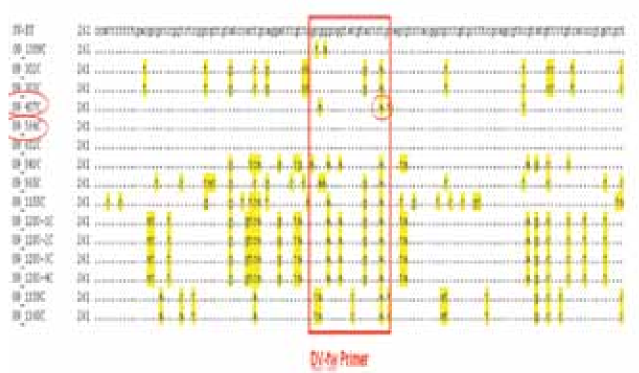
The resulting cDNA was amplified by conventional PCR with the ORF5 primers specific for a 219 bp long fragment within the ORF5 region. After 1.5 % agarose electrophoresis, amplicons were stained with ethidium bromide and visualized under UV light. RT-PCR-products exhibiting specific bands were purified using the GeneJET PCR purification Kit (Fermentas, Germany) and submitted for sequencing (QIAGEN Sequencing Service, Hilden, Germany). PCR products were cloned into the Topo 2.1 vector (Invitrogen, Darmstadt, Germany) and served as templates for *in vitro* transcription.

Results

After cloning the ORF5 gene of Porcilis® PRRS, a tenfold dilution series was prepared starting from 10^{10} copies and amplified by DV RT-PCR. Detection of the gene was possible up to 100 copies, indicating the high sensitivity of the DV-PCR. Same results were obtained for the conventional ORF5 RT-PCR. A checkerboard titration was performed in order to exclude interference between EU field strains and the EU vaccine strain when both are present in the same sample. From these results inhibitory effects could be ruled out.

From the analyses of the PRRS EU positive samples, only two were ambiguous in the DV RT-PCR. Detailed sequence analyses revealed one nucleotide exchange at position 342 in the forward primer binding site (T/A) which led to these uncertain results (Figure 1).

Figure 1. Sequence analysis of the sense primer binding site of the DV specific RT-PCR. Only two isolates show a nucleotide exchange at position 342.



Discussion

It is extremely important to differentiate reliably between PRRSV field strains and the EU vaccine strain because the result of the conventional ORF5 PCR is limited simply to a positive or negative statement. This can lead to confusion for the veterinarian, as well as for the farmer, and should be avoided.

These results show that the DV-PCR is a very potent tool, offering a high degree of confidence. Nevertheless, in cases in which the positive Porcilis® PRRS result fails to accord with the anamnesis and the animals' vaccination regime, further analyses are necessary. Ultimately, sequencing of the fragment would provide the required certainty with respect to the genetic background.

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COMPARISON OF DIFFERENT SAMPLE - MATERIALS FOR THE ABILITY TO DETECT PRRSV IN PIGS USING PCR

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Introduction

Vaccination against Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) leads to a reduction of clinical signs but fails to protect against infection. Vaccines based on a single PRRSV strain are not or only partially effective in protecting against infections with genetically diverse field strains. Sometimes clinical signs related to PRRSV appear in a herd, despite sow vaccination against PRRSV (1). In such case virus isolation is necessary to confirm the PRRSV status of the farm.

The aim of the present study was to compare the ability for the detection of PRRSV-genome using different materials using PCR.

Materials and methods

Eighty patients of the Clinic for Swine of the LMU Munich with PRRSV associated clinical signs were included in this study. A mixture of lung and tonsil tissue (ToLu), Bronchoalveolar-Lavage-Fluid (BALF) and serum from each pig were examined for the occurrence of PRRSV-genome.

Results

Forty four pigs were positive in at least one sample for PRRSV-genome. 17 of these animals were positive for the EU-strain or the US-strain. 10 animals were positive for both strains EU and US (tab. 1).

Table 1. Number and percentage (n=80) of PRRSV positive pigs according to the sample material.

	total	ToLu	BALF	Serum
PRRSV	**44** (55%)	**35** (43%)	**27** (33%)	**28** (35%)
PRRSV only EU	**17** (21%)	**12** (15%)	**16** (20%)	**9** (11%)
PRRSV only US	**17** (21%)	**16** (20%)	**9** (11%)	**17** (21%)
PRRSV EU+US	**10** (12%)	**7** (9%)	**2** (3%)	**2** (3%)

PRRSV was detected most frequently in ToLu (35 out of 44 positive), followed by BALF and Serum (both 27 out of 44 positive). The prevalence and confidence interval is shown in table 2.

The US-strain could be detected significantly more often in ToLu when compared to BALF. The EU-strain could be detected more frequently in serum than in BALF. The ratio of the US-strain in serum compared to BALF was inverse. But these observations were not statistically significant. According to Cohen's Kappa, the measure of agreement between BALF and ToLu (κ= 0.48) as well as between BALF and Serum (κ=0.58) was moderate. The agreement between Serum and ToLu (κ=0.66) was good.

Table 2. Prevalence (%) and confidence interval of PRRSV detection in ToLu, BALF and Serum.

	ToLu	BALF	Serum
total	**79.5** 65.5 - 88.8	**61.3** 46.6 - 74.2	**63.6** 48.8 - 76.2
PRRSV US	**85.1** 76.5 – 94.0	**40.7** 24.5 - 59.2	**70.3** 51.2 - 84.1
PRRSV EU	**70.3** 51.5 - 84.1	**66.6** 47.8 - 81.3	**40.7** 24.5 - 59.2
PRRSV US+EU	**70** 39.6 - 89,2	**20** 5.6 - 50.9	**20** 5.6 - 50.9

Discussion

After intrauterine PRRSV-infection, virus or particles of the virus can be found up to 132 days (2, 3) and up to 157 days when infected as young piglets (4,5). Virus can be found in clinical unsuspicious farms and viremia can still be seen in the presence of neutralizing antibodies (5). Infectious particles and viral RNA are eliminated faster in serum, spleen or lungs than in tonsils (3). Therefore PRRSV field isolates can still be present in a pig herd, although a vaccine is used (4). In the present study the mixture of tonsil and lung has been shown to be the most applicable material to isolate virus genome on a farm.

The results of the study demonstrate the importance of selecting an appropriate material for the detection of PRRSV-genome on a farm.

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DETECTION OF ANTI-PRRSV ANTIBODIES ON ORAL FLUID SAMPLES FROM INDIVIDUAL BOARS USING A COMMERCIAL PRRSV ELISA

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Introduction

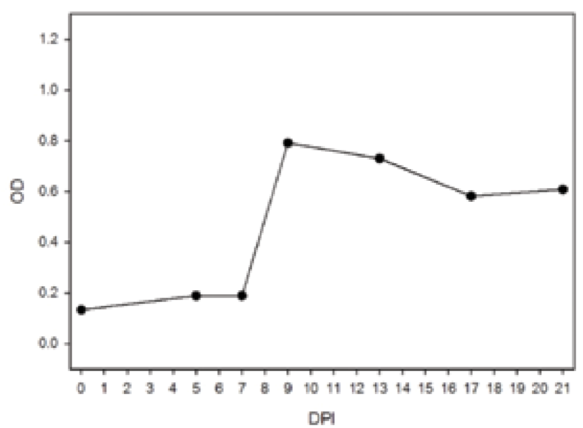
Oral fluid samples are easily collected and their use in surveillance of PRRSV and PCV2 in group-housed pigs under both experimental and field settings is well documented.[1,2] Previously, we reported shedding of PRRSV in oral fluids collected from individually penned boars over the course of 21 days post inoculation (DPI).[3] The purpose of the present study was to determine whether PRRSV infection in individually-housed adult boars could be monitored by measuring anti-PRRSV antibodies in oral fluid samples.

Materials and methods

In 3 trials, 24 boars, 5.5 months to 4 years in age, were intramuscularly (IM) inoculated with a modified-live PRRSV (MLV) vaccine (Trial 1), a Type 1 PRRSV isolate (Trial 2), or a Type 2 isolate (Trial 3). Oral fluid samples were collected daily and serum samples were collected twice weekly. Following the completion of the study, samples were randomized and blind-tested for anti-PRRSV antibodies by using commercial ELISA for the detection of PRRSV serum antibodies (HerdChek® PRRSV X3 ELISA, IDEXX Laboratories, Inc.).

Results

As shown in Figure, anti-PRRSV antibodies were detected at 9 DPI and were detected throughout the sampling period.



Discussion

Although these are preliminary data, these results indicated that the commercial ELISA evaluated in this study can be optimized for detection of anti-PRRSV antibodies in oral fluids. Overall, the data suggested that the detection of anti-PRRSV antibodies in oral fluids may be a useful tool for monitoring PRRSV infection in commercial swine herds (Figure 10). Studies in progress will evaluate the diagnostic performance of the assay in both individual animals and group-housed pigs.

Acknowledgements

This project was founded in part by the PRRS CAP USDA NIFA Award 2008-55620- 19132. Herdchek® PRRS X3 ELISA kits were provided by IDEXX Laboratories, Inc., Westbrook, Maine USA

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P.133
DEVELOPMENT OF IN HOUSE ELISA FOR THE DETECTION OF ANTIBODIES TO PRRSV IN ORAL FLUIDS

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Introduction

Antibody detection in serum by ELISA is the most important method for PRRS diagnosis in swine herds. The correct and useful interpretation of the ELISA results can be made only if multiple samples from pigs from different age groups are collected and analysed. The alternative method of PRRS diagnosis based on detection of antibodies in oral fluids was proposed (1). However, no commercially available ELISA kit is designed for this. The aim of the present study is to present preliminary results on the development of the new in house ELISA for the detection of PRRSV specific antibodies in oral fluids.

Materials and methods

Oral fluids were collected from 2 farms known to be free from PRRSV and 3 farms where PRRSV infection was previously identified. From 60 to 140 serum samples were obtained from each farm that represented pigs from 4 to 23 weeks of age. From each age group 10-25 serum samples collected from animals housed in 2-5 pens. From each pen one oral fluid sample was collected by hanging a piece of rope to which pigs had access for 30-60 minutes. From 12 to 36 oral fluid samples were collected from each farm. The oral fluid soaked piece of rope was cut off and placed in a string bag and transported to the lab where oral fluid was recovered and put in -20°C. For the oral fluid ELISA the antigen (capsid protein expressed in E. coli) was produced and purified as described elsewhere (2). However, modifications of the original ELISA procedure were made: higher concentration of the antigen, lower dilution of oral fluid compared to serum, increased incubation time and increased conjugate concentration. The serum samples were tested with the standard in house ELISA (2). The sensitivity of serum and oral fluid ELISAs to detect antibodies in individual pens (n=64) was compared using McNemar's test.

Results

The specificity of the oral fluid ELISA was evaluated on 52 oral fluid samples from two PRRSV free herds. Only two oral fluid samples from one farm were positive (specificity 96.2%). One sample was from 6 and the other from 20 weeks old pigs. All serum samples from these herds tested negative by ELISA and PCR.

The serum and oral fluid samples obtained from PRRSV infected herds represented age groups between 4 and 20 weeks so they were used to generate PRRSV seroconversion profiles. Comparison of seroconversion in age groups gave nearly identical results with both, serum and oral fluid ELISA (Table 1). There was no significant difference ($p > 0.05$) in sensitivity to detect PRRSV seroconversion in individual pens (Table 2).

Table 1. Profiles of seroconversion in analysed farms. Abbreviations wk – age in weeks, s(+) – number of positive sera in age groups, of(+) – number of positive oral fluids in pens.

Farm 17451			Farm 17452			Farm 17453		
wk	s(+)	of(+)	wk	s(+)	of(+)	wk	s(+)	of(+)
5	0/20	0/4	9	0/15	0/3	4	1/20	0/4
8	16/20	3/4	11	3/15	2/3	7	9/20	3/4
11	16/20	3/4	13	9/15	3/3	9	14/20	4/4
14	19/20	4/4	15	13/25	4/5	13	6/10	2/2
17	18/20	4/4	17	14/20	2/4	15	6/9	1/2
20	17/20	4/4	19	12/20	4/4	17	7/10	1/1
						20	10/10	1/1

Table 2. Results of ELISA analysis of sera and oral fluids in 64 examined pens from PRRSV positive farms.

		Oral fluid		
		Positive	Negative	Total
Sera	Positive	48	2	50
	Negative	2	12	14
	Total	50	14	64

Discussion

The preliminary results indicate that the developed ELISA allows to substitute serum with oral fluid samples for a pen based diagnosis. Collection of oral fluid is relatively easy and the number of tested samples necessary to analyse the detailed profile of seroconversion can be reduced. This method can be convenient, cost efficient alternative to detection of PRRSV antibodies in serum. However, further studies are needed to better assess the test's specificity.

Acknowledgements

The study was supported by the following projects: MNiSW N N308265136, COST FA0902, FP7 245141.

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COMPARISON OF TWO ELISA TESTS FOR THE DETECTION OF ANTIBODIES AGAINST PRRS: INGEZIM DR AND IDEXX X3

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Introduction

Early diagnosis of porcine reproductive and respiratory syndrome virus (PRRSV) is a crucial element of control of this infection. At present, the earliest way to detect a positive animal is by means of RT-PCR using blood samples. If detection of antibodies was feasible and suitable at very early times post-infection, costs of early diagnosis could be substantially lowered. The present report deals with the evaluation and comparison of the two newest commercially available ELISAs to detect PRRSV antibodies: Ingezim PRRS DR and Idexx X3.

Materials and methods

ELISAs. Sera were examined by Ingezim PRRS DR (11.PRS.K0) (Ingenasa) and by Idexx PRRS X3 Ab Test (Idexx Laboratories). Both tests were used as recommended by the manufacturer. Results were expressed as a ratio of the optical density (OD) of a given sample over the OD of the positive control provided by the test (S/P ratio). According to manufacturers, S/P higher than 0.175 for Ingezim PRRS DR and 0.4 for Idexx PRRS X3 were considered as a positive result.

Sera from experimental infections. Sera (n=35) were obtained from five experimental infections of 4-week-old piglets -days 0 to 49 post-inoculation (PI)- with different PRRSV genotype I strains (S1-S6). Selected strains shared from 90.6 to 96% of similarity in protein N. In all cases except one (S3), animals were intranasally inoculated with $\geq 1x10^{5.0}TCID_{50}$/ml. Pigs in S4 were infected with a macerated of lung from an infected pig. *Longitudinal profiling of endemic farms.* A longitudinal serological profile was comparatively performed in two PRRSV endemic farms (n=45 pigs/farm) (4, 6, 8, 10, 12, 15, 17 and 20 weeks of age).

Results

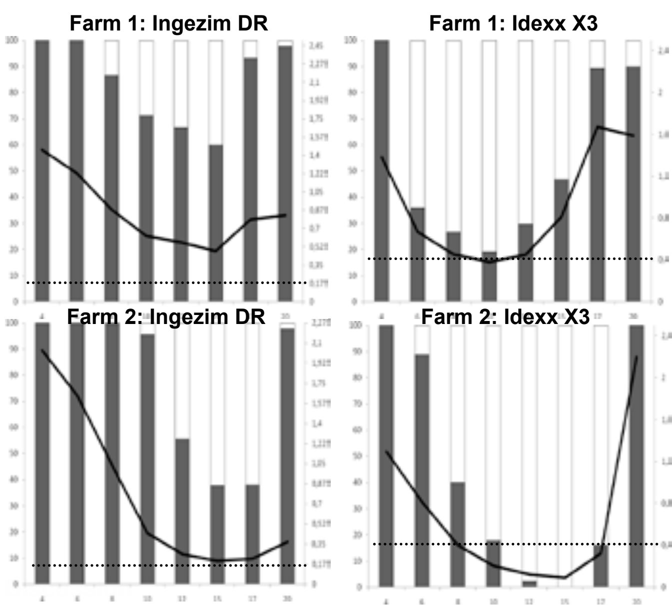
Experimental infections. Table 1 summarizes the percentage of positive pigs in experimental infections until 21 days PI using Ingezim DR or Idexx X3 for each PRRSV strain. Major sensitivity differences were observed at 7 and 14 days PI. All pigs were positive in both tests from day 21 onwards.

Table 1. Proportion of positive sera in each ELISA (N.A. No evaluated).

	Ingezim DR				*Idexx X3*			
Days PI	0	7	14	21	0	7	14	21
Strain 1 (n=5)	0	100	100	100	0	60	100	100
Strain 2 (n=5)	0	60	100	100	0	40	100	100
Strain 3 (n=5)	0	33	84	NA	0	0	67	NA
Strain 4 (n=6)	0	100	100	100	0	17	83	100
Strain 5 (n=7)	0	0	100	100	0	0	100	100
Strain 6 (n=7)	0	100	100	100	0	14	100	100
Total (n=35)	0	69	97	100	0	19	92	100

Longitudinal profiling. Results obtained from farms1 and 2 are summarized in figure 1.

Figure 1. Percentage of positive (grey bars) and negative (white bars) pigs from 4 to 20 weeks of age. Black line expresses the mean of S/P ratio (secondary axis). Black dot line represents the cut-off in both ELISAs.



The examination of experimentally infected animals using the Ingezim DR showed an increased sensitivity in terms of early detection compared to the Idexx X3, probably attributable to the enhanced ability of DR for the detection of IgM (1). When applied under field conditions and because of its enhanced analytical sensitivity, the Ingezim DR recognized as positive piglets with maternally derived antibodies for longer than the Idexx X3 but this also caused some overlapping between the detection of maternally-derived antibodies and antibodies raised after infection of piglets.

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PRRSV NSP7 AS A CANDIDATE TARGET PROTEIN FOR SEROLOGICAL DIFFERENTIATION BETWEEN INFECTED AND VACCINATED ANIMALS (DIVA ASSAY)

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Introduction

Both attenuated and inactivated PRRS vaccines have been developed. The attenuated ones have been proven to be effective with genetically related strains but less effective upon challenge with strains that differ genetically. Recent approaches have focused on the developing of a new generation of inactivated vaccines by improving inactivating protocols. This has allowed the induction of neutralizing antibodies after experimental immunization with these vaccines **(1)**. In order to be able to use vaccines in control programmes, it is desirable to have the possibility to differentiate infected from vaccinated animals (DIVA principle). Non structural protein 7 (nsp7) is relatively conserved within the same genotype (I or II) and its antibody response is measurable from around 10-14 days post infection **(2)**. In this work, the antibody response against nsp7 has been compared as a way to differentiate between infected pigs and pigs immunized with inactivated vaccines.

Materials and methods

Antigen production: nsp7 encoding regions were amplified from a genotype I PRRSV strain by RT-PCR. This sequence was cloned in the pAcHLTA vector and expressed in Sf9 insect cells. Recombinant protein was purified by IMAC and analyzed by Coomasie staining and Western blot.

ELISAs: The nsp7 based ELISA was performed by coating 96-well microtiter plates overnight at 4ºC with purified nsp7 (2,5 ug/ml) diluted in carbonate buffer. After stabilization, plates were incubated 1h at 37ºC with 100ul of pig sera diluted 1:200. After washing, plates were incubated for 1h at RT with horseradish peroxidase conjugated protein A. Finally, after washing, the ELISA was developed with 2,2´-azinobis(3-ethylbenzthiazolinesulfonic acid) (ABTS), stopped with 2% SDS solution and read at 405nm. Cut off was established as mean OD value at 0 days post inoculation (dpi) plus 0,2.

Sera from experimental infection: Sera were obtained from four experimental infections of 14-week-old piglets at 0 to 31-42 dpi with different PRRSV genotype I strains (Lelystad, 07V063, 08V204 and Lena; 6 pigs/group). In all cases, animals were intranasally inoculated with $10^6 TCID_{50}$/pig.

Sera from experimental vaccination: Sera were obtained from three experimental immunizations with inactivated PRRSV and were collected one week after booster vaccination (dpv) with different PRRSV genotype I strains (Lelystad, 07V063 and 08V204; 6 pigs/group).

Results

Antibody levels against nsp7 in infected pig sera tended to be higher for Lelystad and Lena strains. The detection started between 10 and 14 dpi.

For the strains 07V063 and 08V204, OD values reached with post infected sera were low and, when detected, the increase in OD seemed to be a little bit delayed compared with Lelystad and Lena. In the post vaccinated groups, 15/18 sera remained below the cut off.

Discussion

For the pigs infected with Lelystad and Lena strains, there are differences in the levels of antibodies against nsp7 from 26-28 dpi onwards, compared with the post vaccinated group.

For the pigs infected with 07V063 and 08V204 strains, the levels detected in sera from post infected animals are too low to allow differentiation between infected and vaccinated pigs. So far, we do not know the causes of the lower signals reached by pigs infected with these two strains.

The preliminary results presented here indicated that nsp7 could be a good target for setting up a DIVA assay for inactivated vaccines. In any case, further research is necessary to ensure the ability of the assay and to establish the optimal conditions (e.g. mixing of recombinant nsp7 from less homologous strains).

Acknowledgements

The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/ 2007-2013) under grant agreement nº 245141 (coordinator Hans Nauwynck)

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EVALUATION OF LUNG LAVAGE PROTOCOLS FOR RECOVERY OF PULMONARY ALVEOLAR MACROPHAGES IN LIVING PIGS

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Introduction

The standardization of primary porcine alveolar macrophage cultures for PRRSV infection studies is hampered by several factors, e.g. the status of cell activation, genetic background and surface molecule expression patterns. PRRSV infection, replication and pathogenicity in alveolar macrophages were dependent on the expression levels of various receptor molecules, e.g. CD163 and CD169 (1). In addition, a genetically determined difference of pig breeds in the susceptibility towards PRRSV exists, which is essentially due to varying innate immune responses (2). An age-dependent resistance to virus infection was also found (3). For this reason there is need for comparable and reproducible primary macrophage cell cultures and more knowledge about host factors facilitating PRRSV infection. In order to obtain alveolar macrophages from living pigs repeatedly, the applicability of bronchoalveolar lavage was tested in anaesthetized pigs.

Materials and methods

Using a fiberoptic bronchoscope lung lavage fluid samples were taken from the Bronchus trachealis of 32 healthy pigs (German Landrace x Hampshire) approximately 7 weeks of age, originating from a closed herd free of PRRSV. 100 ml 154 mM NaCl divided into five fractions were used as the rinsing fluid.

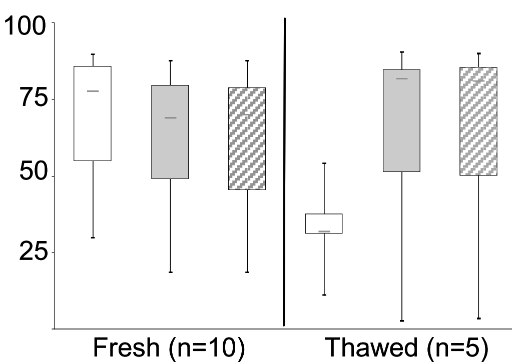
In parallel, modifications of the lavage protocol were tested with respect to the rinsing volume (300 ml), the rinsed lung localization (main lung lobes) and the age of the pigs (postmortal lavage of removed lungs from slaughtered sows).

Living cells prior to and after a standardized freezing process were determined by trypan blue staining. Thawed cells were cultured in a concentration of $2x10^6$ living cells per milliliter in RPMI 1640 medium with 10% fetal calf serum containing Baytril, Kanamycin and Amphotericin B.
Selected bronchoalveolar lavage fluid samples were analyzed by fluorescence-activated cell sorting for the percentage of CD163 and CD169 positive cells.

Results

Postmortal lavage of lungs from slaughtered sows resulted in the highest cell recovery, which enables the culture of PRRSV field strains from organ samples.

In bronchoalveolar lavage fluid samples from the Bronchus trachealis the interquartile range of total macrophage counts was 20.4-30.8 M (median 39.4 M) with 33.5-89.5% living cells. The process of freezing and thawing caused a 30-60% reduction of total cell counts and a 70-90% reduction in living cells resulting in 0.5-8.9 M living cells (interquartile ranges). These thawed primary macrophage cell cultures were viable for 3 days (median) with a minimum of 1 day and a maximum of 28 days. The life-time of cells in culture was highly dependent on the percentage of living cells, which should be approximately 90%. The higher the volume of bronchoalveolar fluid recovery, the higher the total cell counts, the percentage of living cells and the life-time of culture cells. The amounts of cells positive for CD163 and CD169 differed between individuals.



Percentage of living cells ☐ and receptor-positive cells from total amount of living cells: CD163 ▩ CD169 ▨

Discussion

Initial individual differences between fresh cell samples were further increased by processing of cells, resulting in a reduction of living cells, but also in a decreased risk of contamination. Standardized parameters for primary macrophage cultures have still to be elaborated. The susceptibility for PRRSV infection of a standardized primary cell culture could be an indicator trait for susceptibility in vivo (4). A repeated recovery of cells from appropriate donor pigs would be possible using a standardized bronchoalveolar lavage protocol.

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EFFICACY OF THE PRRSV COCKTAIL VACCINES INACTIVATED WITH BINARY ETHYLENIMINE (BEI) IN PIGS

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) vaccine has been used widely in Korea. Two kinds of commercial vaccine was registered and used in Korea. Non formalin based inactivation procedures were employed in this research. Binary ethylenimine (BEI) was used for inactivation of PRRS virus. Previous research presented that BEI could induce favorable virus neutralizing antibodies. This study focused on the efficacy of the BEI-inactivated trial vaccine through the histopathological and virological studies.

Materials and methods

Vaccine Three North American type PRRSV (GC-6262, GC-4019, and GC-DM) and 1 European type PRRSV (GC-EU0907) which were isolated in Korean pig farms. PRRSV were inactivated with 10% BEI for 48 hours at 37℃.

Animals Thirty five 3weeks old piglets which did not have PRRS antibodies.

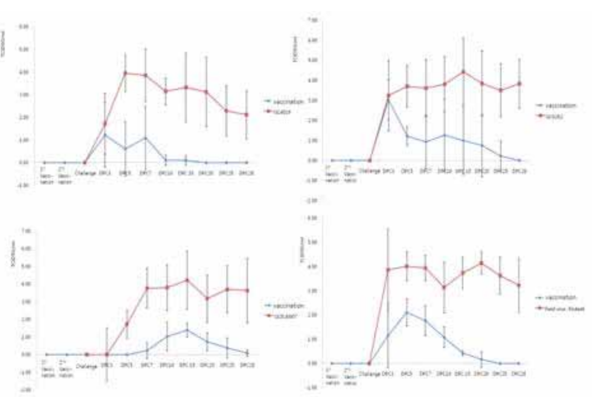
Table 1. Experimental design

	G1	G2	G3	G4
Vaccinated	4	4	4	4
Schedule	*2 shots at 3-week and 5-week old*			
unvaccinated	4	4	4	4
Challenge virus	GC-4019	GC-6262	GC-EU0907	GC-DM
Challenge	*4 weeks after 2nd administration*			
Sampling	D0, 3, 5, 7, 10, 15, 20, 25, 28			
Analysis	Histopathology, virology (viremia)			

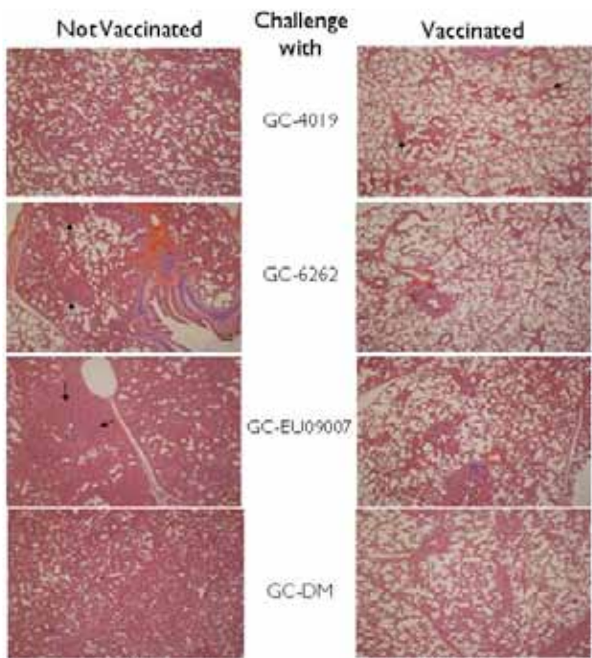
Results

Clinical signs. After challenge with each virus, piglets in non vaccinated groups presented mild fever and anorexia. *Viremia:* Vaccination reduced the duration of viremia and low viral titers compared to non-vaccinated group (Figure 1).

Figure1. Vaccinated groups are significantly reducing viremia. (A) GC-4019 (B) GC-6262 (C) GC-EU0907 (D)GC-DM



Histopathology Microscopic lung lesion of none- vaccinated pigs with 3 NA type and 1 EU type PRRSV infection 28 dpi, showing chronic diffuse mild to moderate interstitial pneumonia. And microscopic lung lesion of vaccinated pigs with each 4 PRRSV strain infection 28 dpi, showing normality but with very mild peribronchiolar and perivascular lymphoid hyperplasia.



Discussion

The BEI-inactivated PRRS vaccine with 4 strains isolated in Korea alleviated the gross and microscopic lesion in lung and reduced the viral shedding after challenge exposure.

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USE OF A FARM-SPECIFIC INACTIVATED VACCINE FOR BOOSTING THE HUMORAL IMMUNITY OF INFECTION-IMMUNE SOWS IN COMPARISON WITH THE CURRENT AVAILABLE COMMERCIAL VACCINES

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a devastating disease causing high losses on reproductive pig farms (1). In the current situation, with PRRSV being widespread in both Europe and the United States, the most appropriate way of handling is to force back the virus through vaccination. In the past, a similar strategy for other viruses (Aujeszky's disease virus, swine fever virus) has proven to be successful. The several vaccines that are available give mixed results regarding the efficacy against the genetically diverse field strains (2). The current attenuated vaccines have problems with stimulating the humoral immune response in infection-immune animals after vaccination. The current inactivated vaccines are safe but low immunogenic, which can lead to no or very limited booster responses. To counter PRRSV, it is necessary to seek the most effective vaccines for sows and piglets. Recently, it was demonstrated in PRRSV-negative animals that by the use of a new procedure, an inactivated PRRSV vaccine could be developed that induces virus-neutralizing antibodies and offers partial protection upon homologous challenge (3). The added benefit is that with this new method farm-specific vaccines can be made. In this study, the booster effect on humoral immunity of this experimental inactivated vaccine in sows with an active immunity after infection will be compared with that of the currently available commercial vaccines.

Materials and methods

The Belgian isolates of PRRSV (n = 3) used in this study originated from unrelated farms showing clinical signs compatible with PRRS in sows or growing pigs. At the moment of sampling, sows of the three herds were vaccinated with the EU-genotype attenuated vaccine (Porcilis® PRRS). From each farm-specific strain (07V063, 08V194 and 08V204) inactivated vaccines were made based on the method described by Delrue et al. (2009). Twenty-five sows, from each PRRSV-positive farm were included in the experiment and transported to the animal facilities of the Laboratory of Virology at the University Ghent. The experimental design was as follows: a first group (n = 5 sows) was a mock-vaccinated control group which received 1 mL RPMI in 1 mL o/w Suvaxyn. Group 2 (n = 5 sows) was vaccinated with 1 mL BEI- inactivated Marc-grown virus (10^8 $TCID_{50}$) in 1 mL o/w Suvaxyn. Group 3 (n = 5 sows) received 2 mL of a commercial European type inactivated PRRSV vaccine (Progressis®, Merial, strain P120: min 2,5 log IF Units). Group 4 and 5 were vaccinated with the European type attenuated vaccine (Porcilis® PRRS, Intervet, 10^4 $TCID_{50}$/2ml) and the American type attenuated vaccine (Ingelvac® PRRS MLV, Boehringer Ingelheim, $10^{4,9}$ $TCID_{50}$/2ml), respectively. All vaccinations in all groups were administered once (single shot) one week after arrival. All sows were monitored clinically. Blood was taken at 0, 1, 2 and 3 weeks after vaccination for determination of both IPMA and virus neutralizing antibodies. Antibodies were detected against the PRRSV strain, originating from the farm where the sows came from. Serum samples collected after vaccination were examined for vaccine virus by titration in Marc-145 cells and pulmonary alveolar macrophages.

Results

All sows remained in good health and condition after they were vaccinated and no local or general reactions were observed. No PRRSV was isolated from any of the samples. In all three set-ups, the strain-specific inactivated vaccine, was the only vaccine that induced both a virus-specific and a strong neutralizing antibody response after booster vaccination, compared to the commercial vaccines, both inactivated and attenuated (European and American type). The efficacy of the commercially attenuated and inactivated PRRS vaccines was dependent on the circulating virus isolate.

Discussion

A farm-specific vaccine, based on the circulating PRRSV field strain can be one of the tools to control PRRSV-related problems. At present, the efficacy of this kind of inactivated vaccine is being tested in the field.

Acknowledgements

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EFFICACY OF A FARM-SPECIFIC INACTIVATED PRRSV VACCINE IN THE FIELD

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Introduction

Porcine and reproductive and respiratory syndrome (PRRS) is the most costly disease currently affecting pig industry worldwide (1). Farmers and veterinarians want to have access to state of the art control measures in order to respond to recent evolutions of the disease. Vaccination of sows is an effective tool to minimize the clinical and economic impact of PRRSV infections. However, recent PRRSV variants have been isolated from herds vaccinated with registered vaccines. In general, the use of a vaccine strain, that is homologous to the prevalent PRRSV-isolate in the herd is advised (2). In this context, a homemade killed vaccine using a farm-specific strain was developed at the Faculty of Veterinary Medicine, Ghent University, Belgium. The purpose of this study was to investigate the booster effect of the inactivated autogenous vaccine in immune sows, and its impact on maternal immunity and on the PRRSV infection pattern in piglets during their first weeks of life.

Materials and methods

The trial has been performed in a commercial all-in, all-out farm, experiencing an endemic PRRSV infection. A fixed number (n = 10) of sows, was vaccinated at 60 days of pregnancy with the PRRSV inactivated autogenous vaccine, based on the isolated strain of that farm. Ten sows were vaccinated with the attenuated European genotype PRRSV vaccine (Porcilis® PRRS, Intervet) at 60 days of pregnancy. Ten sows were not vaccinated and served as control group. Blood was taken from the vaccinated (farm-specific vaccine/ commercial vaccine) and non-vaccinated sows at vaccination, 2 weeks later, and at the beginning and end of the lactation period. Four piglets from each sow were taken blood at fixed time-points (3, 5, 7 and 9 weeks of age). Virus-specific and virus-neutralizing antibody titers were determined using an IPMA-test and SN-test on Marc-145 cells, respectively. Antibodies were detected against the farm-specific strain. Virus titers in serum were determined by virus titration on 24 h cultivated alveolar macrophages (3).

Results

A noticeable increase in both virus-specific as -neutralizing antibodies after vaccination was seen in all sows vaccinated with the farm-specific inactivated vaccine. In the second group, vaccinated with attenuated vaccine virus (Porcilis® PRRS, Intervet), not all sows showed an increase in both virus-specific and -neutralizing antibodies. The sows from the control group showed no changes in both virus-specific and -neutralizing antibodies before and after vaccination. A high amount of antibodies was found till 5 weeks after vaccination in serum of piglets from sows vaccinated with the farm-specific vaccine. The antibody titers of piglets from the Porcilis vaccinated sows decreased faster than those from sows vaccinated with the farm-specific vaccine. Very little or no antibodies were seen in serum of piglets derived from the control sows. The number of viremic piglets from both groups of vaccinated sows was significantly lower than in the control group and the time of appearance of viremic piglets was delayed in piglets from vaccinated animals than in piglets of the control group (see Table 1).

Table 1. Number of viremic piglets in all groups.

	AUTO VACCINE					PORCILIS® PRRS					CONTROL				
	# piglets	3W	5W	7W	9W	# piglets	3W	5W	7W	9W	# piglets	3W	5W	7W	9W
Sow 1	4	0	0	0	0	3	0	0	0	1	4	0	0	3	3
Sow 2	4	0	0	0	0	4	0	0	0	0	2	0	0	0	0
Sow 3	4	0	0	0	0	4	0	0	0	0					
Sow 4	4	0	0	0	1	3	0	0	0	1	4	0	0	3	3
Sow 5	3	0	0	0	0	4	0	0	0	1	4	0	0	0	1
Sow 6	3	0	0	0	1	4	0	0	0	0	3	0	0	1	2
Sow 7	4	0	0	1	2	4	0	0	0	0	4	2	0	2	3
Sow 8	4	0	0	0	2	3	0	0	0	0	4	0	0	2	3
Sow 9	3	0	0	1	1	4	0	0	0	2	4	0	1	2	3
Sow 10	4	0	0	0	0	4	0	0	1	3	4	0	0	0	3

Dis

The farm-specific vaccine can be used next to the commercial available vaccines on farms with PRRSV-related problems to 'stabilize' the immunity status of all breeding animals in order to decrease the transmission of PRRSV from dams to offspring and in between the piglets. Future work will be done to optimize the vaccination schedule.

Acknowledgements

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COMPARISON OF THE EFFICACY OF INACTIVATED PRRSV VACCINE VIRUS GROWN ON MARC-145 OR PK15SN-CD163 CELLS

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) causes reproductive disorders in sows and boars and is associated with the porcine respiratory disease complex (PRDC), resulting in tremendous economic losses (1). In the field, both killed virus and modified live virus vaccines are used to control the disease. PRRSV vaccine virus is currently produced in Marc-cells, because a cell line overcomes problems associated with the use of primary macrophages, the natural host cell of PRRSV. However, since virus entry in Marc-cells is different compared to that in primary macrophages, specific domains associated with virus entry could potentially alter during passages in Marc-cells. Therefore, the $PK^{15Sn\text{-}CD163}$ cell line expressing porcine sialoadhesin (Sn) and porcine CD163, two important receptors for entry and infection of macrophages, the natural host cells of PRRSV, has been introduced for PRRSV production. In this study, the efficacy of a BEI-inactivated vaccine (2) based on 2 recent PRRSV strains, 07V063 and 08V194, grown on $PK^{15Sn\text{-}CD163}$ cells was compared to that based on the same strains, grown in Marc-145 cells.

Materials and methods

Piglets derived from a PRRS-negative farm were used. The following groups were included in a first experiment: a group vaccinated with BEI-inactivated Marc-grown 07V063 virus, a group vaccinated with BEI-inactivated $PK15^{SN\text{-}CD163}$-grown 07V063 virus and a group kept as mock-vaccinated control. In a second experiment, the experimental design was as follows: one group was vaccinated with BEI-inactivated Marc-grown 08V194, the other group was vaccinated with BEI-inactivated $PK15^{SN\text{-}CD163}$-grown 08V194, a third group served as mock-vaccinated control. For both experimental set-ups: all groups were vaccinated twice intramuscularly at 5 (primo vaccination) and 9 (booster vaccination) weeks of age. All animals were challenged intranasally with $10^{6.0}$ $TCID_{50}$ of the virulent virus field strain 07V063 or 08V194 at the age of 13 weeks. Sera were collected by jugular venipuncture at 0, 1, 2, 3, 4, 5, 6 and 7 weeks after primo vaccination and at 0, 1, 3, 5, 7, 10, 14, 21, 28 and 35 days post challenge. Virus-specific and virus-neutralizing antibodies were determined against the PRRSV strain, with which the pigs had been challenged by an IPMA- and SN-test, respectively.

Virus titers in serum were determined by virus titration on pulmonary alveolar macrophages.

Results

Vaccination with BEI-inactivated Marc-145 grown virus and BEI-inactivated $PK^{15Sn\text{-}CD163}$ grown virus resulted in a similar significant reduction of viremia upon challenge.

Figure 1. Viremia in all pigs challenged with 07V063.

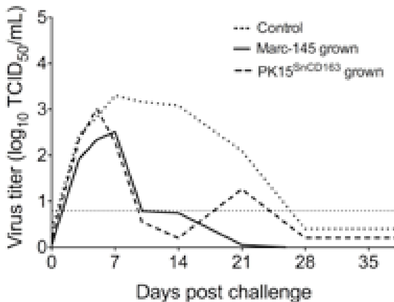
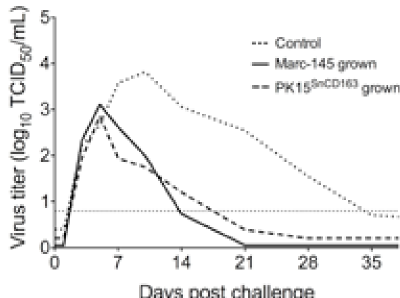


Figure 2. Viremia in all pigs challenged with 08V194.



Discussion

The protection with both inactivated vaccines is comparably effective but still partial, thus it remains a challenge for the future to improve inactivated PRRSV vaccines.

Acknowledgements

This work was funded by the Federal Public Service Health, Food chain safety and Environment (RT - Theme 7 – Acron. PORRS) and the European Union (Seventh Framework Programme; Project No. 245141; coordinated by Dr. H.J. Nauwynck).

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DESIGN OF TGEV DERIVED VECTORS AND ANTIGENIC STRUCTURES TO PROTECT AGAINST PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

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Introduction

A set of coronavirus-derived vectors, based on transmissible gastroenteritis virus (TGEV) was constructed. These vectors expressed PRRSV M protein and GP5 mutants with altered glycosylation patterns, since it has been proposed that removal of the glycosylation sites could lead to the improvement of the immune response against PRRSV (2). Vaccinated animals showed a clear humoral response against PRRSV GP5 and M proteins. Nevertheless, the immune response elicited by these vectors did not provide full protection (3). Two hypotheses may explain the moderate protection obtained. A certain degree of instability in GP5 expression by rTGEV was observed, resulting in a significant lost of GP5 expression with vector passages in cell culture. The lack of full protection using rTGEV expressing PRRSV antigens could also be due to the presence of domains inducing negative regulatory T cells (Treg) in the expressed proteins (4).

Materials and methods

rTGEVs expressing PRRSV antigens were engineering using an infectious TGEV cDNA cloned as an artificial bacterial chromosome (BAC) previously obtained in our laboratory (1). PRRSV ORF5 ectodomain (GP5-ecto) and ORF6 were cloned in the place previously occupied by non-essential 3a and 3b genes. The expression levels and stability of these rTGEV vectors was evaluated by RT-PCR and immunofluorescence assays. Expression of GP5-ectodomain fragment was detected with a monoclonal antibody specific for HA tag, and TGEV was detected with a specific rabbit antiserum. The percentage of infected cells expressing PRRSV antigens was estimated by the analysis of >200 infected cells.

Results

The 68 most N-terminal aminoacids of the GP5 protein, which cover the ectodomain of the protein, were included in rTGEV vectors. To allow GP5-ectodomain protein detection, a hemaglutinin (HA) tag was fused to GP5 protein fragment. Two vectors were generated, with the HA tag located at the amino (GP5-ectoA) or carboxy (GP5-ectoB) terminus of GP5 ectodomain, respectively. GP5 ectodomain fragments were co-expressed with M protein, since it has been previously reported (3) that co-expression of GP5 preliminary results showed that GP5-ectoA and GP5–ectoB were expressed in 93 % and 25% of the rTGEV infected cells, respectively. with M protein increased the stability of GP5 in the vector. After 12 passages in tissue culture,

Previous studies in the laboratory showed that, after 8 passages in tissue culture, full-length GP5 protein was expressed in 85 % of the infected cells. Therefore, expression of GP5-ectoA domain represents a significant improvement of rTGEV vector stability. The protection conferred by these vectors will be evaluated in vivo. In order to increase GP5 stability in rTGEV vectors, viruses expressing GP5-ecto were engineered by scanning mutagenesis that replaced most aminoacids of the GP5-ecto by alanine. The goal is the expression of GP5-ecto domain variants containing the epitopes relevant in protection, but lacking domains responsible for the instability of the heterologous gene.

Discussion

Vector- based vaccines could represent and advantage to stimulate both humoral and cell immune responses against PRRSV. Nevertheless, the results reported to date using viral vectors are not fully satisfactory and new vectors must be explored. The TGEV-based vector has the capability of expressing high levels of heterologous genes, is a potent interferon-α inducer, and presents antigens in mucosal surfaces, eliciting both secretory and systemic immunity. Nevertheless, as reported for other RNA viruses, data obtained indicate that heterologous protein expression stability was limited. New antigenic structures, based on GP5-M platform, were designed and expressed with rTGEV vectors. These structures improved previous constructs stability and may also increase protection against PRRSV by the elimination of negative signals for the immune system (Treg)

Acknowledgements

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HERD BASED PRRS RISK INDEX TO EXPLORE ERADICATION PERSPECTIVES IN PIG DENSE AREAS IN NORTHWESTERN GERMANY

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Introduction

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is widely distributed in the German pig population with an estimated herd prevalence of 85 to 90%. The perspectives of a nationwide PRRSV eradication have been intensively discussed but the outcome is difficult to predict. Eradication from herds is achievable using various strategies, but the cost-effectiveness of such efforts depends on the risk of reintroduction of the virus once it has been eradicated (1). This risk is determined by risk factors like herd characteristics (herd structure, biosecurity level, etc.) (2) or neighborhood conditions (proximity to other pig herds) (3).

Our study scores risks for pig herds in two pig-dense counties in Northwestern Germany of staying endemically infected or of getting re-infected under the assumption of the virus being eradicated on a regional level. By this means we want to provide an exemplary database allowing an estimation of the feasibility of a sustainable PRRSV eradication for herds and regions. It allows us to geographically locate areas with minimal or reasonable chances of maintenance of a PRRS-free status when undergoing eradication.

Materials and methods

Anonymized data of two Lower Saxonian counties were obtained from their veterinary administrations:(A) with a very high pig density (1700 pigs/km^2); (B) with a medium pig density (220 pigs/km^2) .

Datasets included exact animal numbers as reported by the farmers to the reimbursement fond for epizootic diseases (Tierseuchenkasse), as well as geo-reference data. Geographic analysis was done using the official animal disease reporting software used by German veterinary authorities, Tierseuchen-Nachrichtensystem (TSN 3.0). Attributing weighting factors to the compiled herd characteristics (Table 1), an index was calculated for each herd, relating to its respective risk of endemic virus circulation in the herd and (re)infection during and after virus eradication.

The risk scoring was based on knowledge from literature and expert opinion and risks were translated into estimated factors. The resulting indices were set into a proportional scale, comparing them with a reference herd.

Results

We analysed 1737 (A) and 679 (B) pig herds. For every herd, key features were compiled (Table 1).

Table 1. Herd characteristics listed in the database.

Individual properties	*External properties*
Epidemiological unit (contacts/management)	No. of neighboring herds in a 250 m-radius
No. of suckling piglets	No. of neighboring herds radius 250 -500 m
No. of weaners	No. of neighboring herds radius 500 m -1 km
No. of fattening pigs	No. of neighboring herds radius 1 km -2 km
No. of sows	
Herd type	

Using these herd properties, an overall index for each herd (**R_{herd}**) was generated: the higher the index, the greater the risk for the herd to stay endemically infected or to get re-infected during/following eradication.

Analyzing the geographical distribution of the indexed herds, we identified regions with comparably high risk of (re) introduction and endemic circulation of the virus as well as "lower risk" regions with better chances of a sustained success of eradication.

Discussion

The PRRSV herd prevalence in the observed regions is at an estimated 95%. Eradication is the subject of enduring discussions among several stakeholders but so far lacking data to substantiate arguments for or against its feasibility. With this database, we provide a first overview by mapping relative feasibilities, using objective herd criteria like herd size and neighboring herds. On this basis, suggestions on implementation of eradication measures can be developed and possibly get extended to other regions in Germany.

Next steps could be the synchronization of our data with trade network data to integrate this further factor. It has to be underlined that fine-tuning of the model assumptions is an ongoing process, transforming it with every new input.

Acknowledgements

To S. Tapper & J. Diekmann (LAVES); R. Schröder (FLI) and the participating veterinary administrations

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AN ATTEMPT OF PORCINE REPRODUCITVE AND RESPIRATORY SYNDROME (PRRS) ELIMINATION WITH NATURAL EXPOSURE ON A SMALL PIG FARM IN SLOVENIA

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is causing a significant economic impact on the swine industry worldwide. Several management protocols have been used to control PRRSV with varying degrees of success (1). There is only restricted cross protection against challenge with heterologous strains of PRRSV (2). A variety of strategies has been described for PRRS eradication: total depopulation/ repopulation, partial depopulation, segregated early weaning, test and removal, mass vaccination and herd closure for spontaneous virus spreading (3, 4).

The first and the most important are to evaluate whether we can keep the system negative during and after the eradication/elimination program. The second step is to create an infected, recovered and immune population of reproductive animals. To reach this aim the closure of the farm from the introduction of replacement animals is necessary (3). Vaccination, serumization or spontaneous natural spreading can achieve a simultaneous immunity of the breeding herd (4). The aim of the study was to prove the effectiveness of PRRSV elimination by natural spreading of PRRSV on a small pig farm in Slovenia

Materials and methods

Farm
The study was conducted between September 2009 and November 2010 on one site pig farm with 50 sows and 3 boars, free of classical swine fever and Aujeszky disease. After confirmation of PRRSV, farm was closed in October 2009.

Samples
In total 155 serum samples were taken in 7 samplings for antibody detection (Table 1) and 46 serum samples in three samplings (15 samples on 25.09.2009, 9 samples of pools from 43 sows on 01.04.2010 and 22 samples on 11.10.2010) for PRRSV antigen detection.

Methods
155 serum samples were tested with IDEXX PRRS ELISA (HerdChek, IDEXX Laboratories Westbrook, Maine, USA). Results were expressed in S/P (sample: positive) ratios (**L** – Low S/P ratio less than 2, **H** – High S/P ratio more than 2).

Forty-six samples were screened with one step RT-PCR (Qiagen, Germany) and specific primers for detection of EU/NA PRRSV in highly conserved region of ORF 7 (5) and two PCR positive samples (detected in October 2009 and October 2010) were subjected to direct sequencing.

*Records of weaner succumbs and culls.*Number of succumbed and culled weaners was recorded weekly from October 2009 until April 2010.

Results

Table 1. ELISA results

Sampling		***No. of samples***	Results (S/P)	
			L	**H**
1	11.09. 2009	9	1	8
2	25.09. 2009	15	1	14
3	01.12. 2009	53	53	0
4	01.04. 2010	43	21	22
5	07.07. 2010	5	3	2
6	22.09. 2010	8	0	8
7	11.10. 2010	22	4	18

By RT-PCR, PRRSV was detected in 3, 0 and 3 samples out of 15, 9 and 22 tested samples respectively. Sequences comparisons of detected PRRSV in September 2009 and October 2010 revealed only 90 % identity (comparison on 258 nt of ORF 7), confirming the second introduction of PRRSV on farm.

In October 2009 92 succumbs and culls were recorded and the number of pig losses was decreasing until February 2010 to 27. Later in March and April 2010, losses started increasing again to 32 and 34.

Discussion

The positive effect of the herd closure was evident after three months by serological testing and with decreased number of succumbed and culled as well as with sows negative for PRRSV in April 2010. Therefore, we expected negative fatteners, but results of ELISA revealed that they were H positive and later new strain of PRRSV was detected in the herd. Conclusion from the study is that after successful elimination of the virus, new strain of PRRSV was introduced.

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ASSESSMENT AND VERIFICATION OF THE EFFICACY OF THE SIMULTANEOUS USE OF PORCILIS M HYO AND PORCILIS PRRS IN A FIELD STUDY

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Introduction

Worldwide, the interaction of PRRSV and *Mycoplasma hyopneumoniae (M. hyo)* causes respiratory diseases in combination with lung lesions. As supported by previous laboratory studies (1), the simultaneous vaccination against both agents provides a reliable protection against M. Hyo caused pneumonia.The objective of this study was to assess and verify the efficacy of the simultaneous use of Porcilis M Hyo and Porcilis PRRS under field conditions.

Materials and methods

The field trial was conducted on a piglet producing farm and the following wean-to-finish farm in Austria, which reported respiratory problems associated with *M. hyo* and PRRSV. In total, 1000 one week old suckling piglets were divided into five groups. They were either vaccinated against *M. hyo* and/ or PRRSV or not vaccinated (Table 1). Blood samples were taken from 15 % of the pigs from each group, at seven specific time points (1st, 3rd, 6th, 10th, 15th, 20th and 24th week of life). The samples were analysed serologically for the presence of antibodies against PRRSV (data not shown) and *M. hyo.* by ELISA (Ingezim M Hyo Compact, Ingenasa, Immunologia y Genetica Aplicada S.A.,Madrid, Spain). An individual lung scoring (M. hyo-induced lung lesions (2)) was conducted at the slaughterhouse.

Table 1. Vaccination schemes of the different groups.

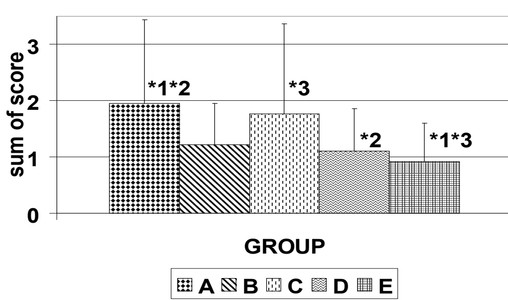
Group	Product:	1st week	3rd week
A	Porcilis PRRS		X
B	Porcilis M Hyo	X	X
C	Diluvac Forte (Placebo)	X	X
D	Porcilis PRRS		X*
	Porcilis M Hyo	X	X*
E	Porcilis PRRS		X**
	Porcilis M Hyo	X	

* Concurrent use: vaccines are administered at the same time, but at different injection sites. ** Simultaneous use: lyophilised Porcilis PRRS dissolved in Porcilis M Hyo is administered in one injection at one site.

Results

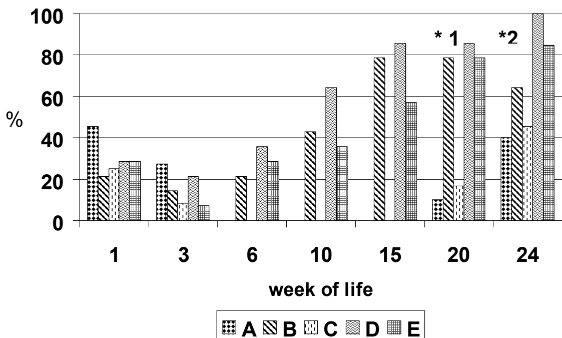
Significant differences could be observed in the lung lesion score of the affected lungs from the different groups (Figure 1) as well as in the serological results (Figure 2). The lung lesion scores of the piglets of all three Porcilis M Hyo vaccinated groups were lower than the scores of the non Porcilis M Hyo vaccinated groups. In the dual vaccinated groups(D and E) the percentage of M. hyo antibody positive pigs was higher in week 20 and 24 than in the non *M. hyo* vaccinated groups (Figure 2) No significant differences could be observed between the groups regarding the clinical parameters.

Figure 1. Mean values plus standard deviation of the severity code of affected lungs of the different treatment groups.



*1 p=0.006 (A:E), *2 p=0.02 (A:D),*3 p=0.022(C:E)

Figure 2. Percentage of M. hyo. antibody positive pigs of each study group at specific time points.



*1 p=0.001 (A:B), p=0.001 (A:D) p=0.001 (A:E), p=0.002 (B:C), p=0.001 (C:D), p=0.002 (C:E) *2 p=0.039 (A:E)

Discussion

The obtained findings verified previous laboratory results under field conditions (1). The lungs of the pigs of the simultaneous vaccinated group were affected the least by the M. Hyo infection and yielded even a lower lung score than the lungs from the concurrent vaccinated group. Therefore, it can be concluded that the simultanous use of both vaccines had a positive influence on the lung health. It could be assumed that the PRRS vaccine had a synergistic effect on the local immunity in the lung tissue (3).

Acknowledgements

This work was supported by Intervet/Schering-Plough

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THE EFFECT OF THE VACCINATION OF TWO-WEEK OLD PIGLETS WITH PORCILIS® PRRS ON POST-WEANING MORTALITY RATE

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Introduction

For some years it has become customary to live with PRRS disease because of the impossibility of eradicating it. The term 'positive but stable farm' is now in routine use, meaning a farm which, despite the presence of PRRS antibodies in the sows, cannot be guaranteed to produce uninfected piglets (1). On many occasions, in transitions from positive but stable farms, seroconversion is detectable after the disappearance of the maternally derived antibodies, and PRRS virus can be found by PCR in animals already weaned.

The aim of this study was to evaluate the benefits of the vaccination of piglets during the second week of life with Porcilis® PRRS (an attenuated European strain vaccine), based on the reduction in mortality rate.

Materials and methods

The trial was performed in 2010 on a farm with two production centres separated by 100 metres. The centres, Origin 1 and Origin 2, contained 600 and 900 sows, respectively. Piglets were weaned at 25 days of age, and passed onto an Isowean unit situated 25 km away. The Isowean was subdivided into areas which were filled with animals from each centre, alternately.

All the sows were seropositive for PRRS and PCR negative. The piglets from Origin 2 were seropositive on weaning (3 weeks of age) and PCR negative, while the piglets from Origin 1 were seropositive and PCR positive. In the Isowean the animals seroconverted positive against PRRS at 6 weeks of age. In the necropsies, lesions compatible with viral pathology were detected, together with those produced by *Haemophilus parasuis* and *E.coli*.

None of the piglets were vaccinated, and those in this initial situation were designated Batch A. Following analysis of the clinical history, it was decided that for another batch, Batch B, the piglets from Origin 2 (B2) would be vaccinated, and for another, Batch C, all piglets from both sources (C1: N=2,000; C2: N=3,000) would be vaccinated. SPSS (2006) 15.0, IL, USA was used for the statistical analysis.

Results

Table 1 summarises the mortality and the probability of survival observed on the farm through various breeding cycles, prior to vaccination (Batch A), and after partial (Batch B) and complete vaccination (Batch C). Mortality rate decreased in Batches B and C. Comparing the Kaplan-Meier survival estimation graphs, it can be seen that in Batch B there is an overall decline in mortality which follows a similar pattern in both groups, with no significant difference between them.

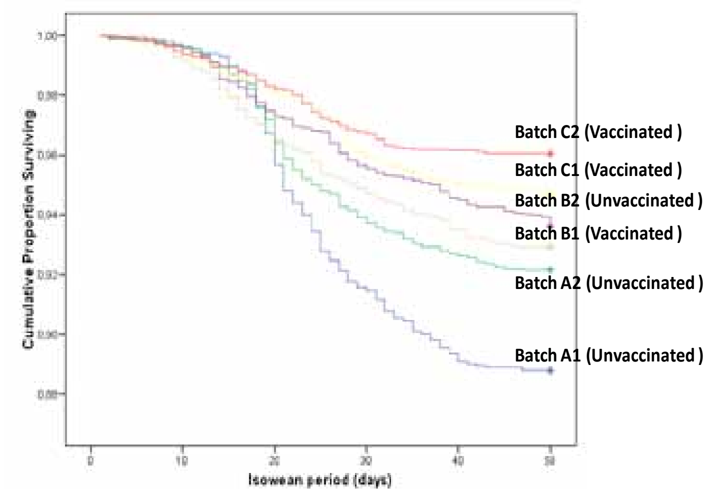
By contrast, the reduction in total mortality in Batch C is greater, and with a statistically significant difference (p=0.026) between groups, the rate being better in the group of piglets from Origin 2.

Table1: Mortality results and probability of survival

	Mortatilty (%)	Probability of survival (%)	Sig(*)
Batch A			
Origin 1 (Unvaccinated)	11.2	88.8	***
Origin 2 (Unvaccinated)	7.8	92.2	
Batch B			
Origin 1 (Vaccinated)	7.1	93.8	NS
Origin 2 (Unvaccinated)	6.4	93.6	
Batch C			
Origin 1 (Vaccinated)	5.3	94.5	*
Origin 2 (Vaccinated)	4.0	96.0	

*NS: No significant ; * p<0.05 ; ***p< 0.001 (Log-rank test)*

Figure 1: Survival during the Isowean phase in piglets from Origins 1 and 2 in three different batches (A,B,C).



Discussion

It is well established from field and experimental studies that vaccination against PRRS in growing pigs is effective (2,3). In the present trial, it has been shown to be a very efficient tool for improving post-weaning mortality rates in piglets vaccinated during the second week of life. It should be noted that the trial was conducted with a very common system of production that is, one in which piglets from different sources, and of varying health status in respect of PRRSv, are mixed for the growing phase of their development.

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EFFICACY OF VACCINATION AGAINST PRRS VIRUS FOR REDUCING VIREMIA AND CONTACT TRANSMISSION

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Introduction

If a vaccine cannot produce sterilizing immunity, the key element for controlling a disease through vaccination will be the increase of the minimal infection dose for vaccinated pigs and the decrease in the potential of vaccinated but infected animals for transmitting the infection. As a result, the reproduction rate (R_0) of the infection would be close below or close to 1 and the infection will extinguish. The present study was aimed to determine the outcome of infecting vaccinated pigs with heterologous PRRSV isolates and mixing those pigs with vaccinated and unvaccinated sentinels.

Materials and methods

Pigs and facilities: 31 high health 3-week old piglets from a PRRSV-free farm were allocated in two different pens: A (n=20) and B (n=10). Group A was vaccinated with a commercial vaccine (Porcilis® PRRS; Intervet-Schering Plough) and group B received a placebo. At day +42 post-vaccination (PV) pigs were randomly re-grouped: 7 A piglets were mixed with 4 B ones (group Ch1); other 7 A piglets were mixed with 3 B ones (group Ch2) and allocated in a second pen. Three more A pigs were mixed with 1 B pig (group T1) and allocated in a pen in front of group Ch1. Similarly, 3 A pigs and 2 B ones were mixed (T2) and allocated in front of Ch2. Pens Ch and T were separated by corridor of 1 m wide. Pens had open partitions, the air space was common. Temperature and humidity were in the range of 22±5ºC and 50-75%, respectively.

Ch1 and Ch2 pigs were intranasally inoculated respectively with 2 ml ($5x10^5$ $TCID_{50}$/ml) of PRRSV strains S1 and S2 (isolated from abortion outbreaks, <93% similar to the ORF5 of the vaccine). T1 and T2 were kept as controls. Pigs were bled at days 0, +21 and +42 PV and at days 0, 3, 6, 14 and 21 post-challenge (PC). PRRSV antibodies were tested by ELISA (Idexx) at days 0, +21, + 42 and at days 0, 3, 6, 14 and 21 PC and a viral neutralization assay was performed at days 0 and +21 PC. Isolation of the virus was done in porcine alveolar macrophages. To further assure negative results, serum samples were also tested by nested RT-PCR (nRT-PCR) (1). Vaccine efficacy was calculated from the etiologic fraction.

Results

Vaccination resulted in seroconversion of pigs but with no development of detectable neutralizing antibodies before challenge. After challenge unvaccinated pigs in Ch1 and Ch2 seroconverted. Regarding virological examinations, all unvaccinated animals in Ch1 and Ch2 became viremic after challenge and this state lasted 14 to 21 days. In contrast, the proportion of viremic animals in vaccinated pigs was much lower (Table 1). No statistical differences were found between S1 and S2 groups and, accordingly, were treated as a whole.

Table 1. Number of viremic pigs after challenge as determined by isolation and titration in macrophages.

	Days post-challenge				
	0	**+3**	**+6**	**+14**	**+21**
Unvaccinated	0/7	7/7*	6/7*	5/7 *	1/14
Vaccinated	0/14	3/14	2/14	0/14	0/14

*p<0.05

Using nRT-PCR, at +21 PC, 7/7 unvaccinated pigs and 5/14 vaccinated animals were positive (p<0.05). Efficacy of vaccine for preventing viremia was 71.4%. Unvaccinated animals in groups T1 and T2 remained seronegative, indicating that transmission of the infection did not take place between pens separated by 1 m of air space.

Discussion

The present study shows that vaccination against PRRS substantially reduced both the proportion of viremic pigs and the duration of viremia. Furthermore, the results indicated that unvaccinated and inoculated pigs were not efficient to transmit the infection, and most vaccinated contacts withstand both the inoculation and a 21-day contact with infected animals. Moreover, sentinel animals allocated just one metre far from inoculated pigs could not be infected by sharing the air space. The fact that some animals were found to be negative in viral isolation but positive in nRT-PCR most probably reflects the ability of the PCR for detecting non-viable virus. The present results suggest that the epidemiological premises for controlling PRRS through mass vaccination could be fulfilled.

Acknowledgements

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A SAFE AND EFFICACIOUS MLV VACCINE CAN CONTRIBUTE TO THE CONTROL AND ERADICATION OF PRRSV

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Introduction

PRRSV infections still have a major impact on pig production, even though vaccines have been in use for more than 15 years. Inactivated vaccines have a limited efficacy (1,2) but, in contrast, Modified Live Virus (MLV) vaccines have been shown to be effective in the main. In the US, stabilization and subsequent eradication have been achieved largely by herd closure and partial depopulation (3), the basic concept being to prevent the presence of susceptible animals. All incoming gilts are exposed to PRRS field or vaccinal virus.

A major concern with respect to MLV vaccines was, and still is, a particular aspect of their safety: the vaccinal virus of all MLV PRRS vaccines spreads, at least to some extent (4).The risk with these vaccines, especially the potential to revert to virulence (which actually occurred with a US strain MLV vaccine), has long been the subject of discussion.(5) This paper aims to show that not all MLV vaccines are similar in this respect.

Materials and Methods

In Denmark, a trial was carried out to measure the spread of vaccinal virus between sows. Nine pens of weaners (78 in total) were vaccinated with Porcilis® PRRS, a MLV vaccine based on an EU strain. Two other pens of weaners located between these pens, were left unvaccinated to provide sentinel piglets. Also, 15 sentinel unvaccinated sows were housed in the same building but without direct (nose to nose) contact with the piglets. Blood samples were taken from the sows six times at 3-weekly intervals (6).

Five further trials were undertaken to determine the transmission of Porcilis PRRS, in two of which the spread was compared to that of another PRRS MLV vaccine based on the US strain. Some piglets were vaccinated and some left as sentinels, and blood samples were taken regularly from all of them up to 9 weeks after vaccination. The samples were checked for PRRS antibodies, And the transmission ratio (R0) was calculated from the number of seropositives.(R0 is the number of animals that will be infected by a single infected animal).

Results

The Danish trial showed that all vaccinated piglets showed serovonversion in a PRRS ELISA. Only four sows seroconverted and a single piglet of the sentinel groups. Virus was isolated from 23 samples of vaccinated pigs and one sentinel sow taken 3 weeks post-vaccination (and in only one piglet 6 weeks after vaccination.) Based on these data, R0 was calculated to be 0.06 ±0.09 (6). The results from the five comparative trials are shown in Table 1.

Table 1 Transmission experiments in weaners
Vaccine Trial Piglets Serocon

Vaccine	Trial	Piglets		Seroconverted sentinels	R0
		Vacc	Senti-nel		
Porcilis PRRS	1	10	4	2	0.20
	2	60	11	2	0.04
	3a	30	6	1	0.03
	4	6	6	1	0.10
	5a	16	24	2	0.10
PRRS US strain	3b	30	6	5	>0.3
	5b	16	24	8	0.40

For trial 5, the chance of R0 > 1 was estimated. For Porcilis® PRRS this was calculated to be 0.001%, and for the PRRS MLV US strain vaccine to be 10%, with the consequent possibility of virus spreading through a pig population (7).

Discussion

These trials showed that the modified virus of both vaccines does indeed spread to sentinel pigs but to different extents. On a Lithuanian farm, a US strain MLV vaccinal virus circulated for at least 3 years after vaccination (8). In the field, in Germany where both PRRS-MLV vaccines are used, substantial differences have been found between them (9). If MLV vaccines are to be used in programs intended to eradicate the PRRS field virus from a farm, or group of farms, it is extremely important that the vaccinal virus does not add substantially to the amount of field virus in circulation.

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ECONOMIC COMPARISON BEFORE AND AFTER THE USE OF A LIVE PRRS VACCINE (AMERVAC® PRRS)

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Introduction

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) continues to be one of the most economically important swine diseases. In the U.S.A., acute economic impact attributable to PRRSV was estimated at $67 million for breeders and $493 million for growing pigs[1]. The losses were due to decrease reproductive performance, increase mortality and compromised rate and efficiency in growth. In this study, the objective is to assess the economic impact of vaccination using a live PRRS vaccine (AMERVAC®PRRS).

Materials and methods

A 3,000 sow level farm located north of Manila had complaint of abortions and high mortality in nursing and growing-finishing pigs in 2008. PRRSV diagnosis was carried out with PCR[3] and an ELISA serology technique (CIVTEST™suis PRRS E/S) from necropsy findings and serum samples. The farm implemented in October 2009 two mass vaccinations in sows with 4 weeks interval. Regular program of mass vaccination every 4 months was implemented in sows. Pigs aged 18-30 days were also vaccinated once at the same time. Incoming batches of piglets were vaccinated once at 25 days of age. Using a commercially available record keeping file **(PIGCOM®)**, profit was compared before vaccination (October 2008 to October 2009) and after vaccination (October 2009 to October 2010).

Results

A reduction in pig mortality during the whole growing period, (from birth to market weight) was observed during the vaccination period (October 2009 to October 2010). Additional savings of Php 3,615.73 ($80)/sow/year was realized with the improvement in birth to market mortalities.

Table.1. Pig mortality (birth to market weight) before and after vaccination

Period	Total Pigs	Mortality (heads)	Mortality (%)
October2 008-October 2009	12, 060	4,872	40.40
October 2009 -October 2010	12,024	992	8.3

Table 2. Cost-benefit Analysis Before and After AMERVAC®PRRS vaccination

Mortality Difference (heads)	**3874**
Margin per head sold (Php)	**2, 800.00**
Annual Realized Savings (Php)	**10, 874, 200.00**
Additional Savings/sow/year (Php)	**3, 615.73**

***$1 = Php 45.00**

Discussion

As in previous studies vaccinated sows[2,3] were farrowing probably piglets that showed better health status and consequently lower mortalities from birth to market. This has a positive effect in terms of total farm economic situation.

Conclusions

It seems that a regular vaccination program with AMERVAC®PRRS in sows and pigs reduced the negative impact of field PRRSV infection and consequently improved the farm profit.

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DIETARY CONJUGATED LINOLEIC ACID AND INFLAMMATORY RESPONSE OF PIGS TO PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME INFECTION

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Introduction

Conjugated linoleic acid (CLA) is a mixture of geometric and positional isomers of linoleic acid which have beneficial health properties, including anti-inflammatory effect (1-3). This effect has been seen in different animal models. However, its effect is unknown in pigs infected with porcine reproductive and respiratory syndrome virus (PRRSV), a disease that causes significant economic losses in pig production sector. Thus, the aim of this study was to evaluate the effect of dietary CLA on production inflammatory cytokines IL-1β, IL-6 and TNF-α in serum from pigs infected with PRRSV

Materials and methods

The experiment was carried out on Animal Metabolic Unit at CIAD in Sonora, Mexico. A total 15 pigs of 5 wks of age were used (5 per treatment) and assigned to one of the following treatments: CLA 0%, CLA 1% and CLA 2%. The CLA (LUTALIN® BASF) was supplemented for six weeks. Serum samples were collected at the second week of the supplementation (day 0) and at day 7 post-infection (PI) and at 28 PI. The pigs were infected using the American strain NVSL-97-7895 at day 0. The production of cytokines IL-1β, IL-6 and TNF-α in serum was quantified using a commercial ELISA kit according to the manufacturer`s recommendations (BioSource). Also, viral loads were evaluated (viral particles/ml of blood) by real time PCR (Tetracore). Data were analysed by ANOVA using NCSS 2001.

Results

Figure 1A shows that the production of IL-1β was affected by dietary CLA. Before the pigs were infected with PRRSV (day 0) the production of IL-1β tended to decrease (P = 0.058) in the group fed CLA 2% compared with non- supplemented group (CLA 0%), while at 7 pi levels of this cytokine were not affected significantly between treatments. IL-6 did not show significant changes for any treatment at day 0, but once infected (day 7pi) production decreased significantly in pigs supplemented with 1 % CLA compared to pigs fed diets without CLA (Figure 1B). Figure 2 At day 0, before pigs were infected with PRRSV, pigs fed CLA 1% produced more TNF-α (P<0.05) compared with the group CLA 0%. This significant increase in TNF-α showed by CLA in the week prior to infection was also observed in the first week post-infection, but now in both CLA concentrations (1 and 2%). However, at day 28pi, only 2 % CLA diet significantly increased production of TNF-α compared to the pigs fed diets without CLA. Viral load did not show changes during the study.

Figure 1. Production of pro-inflammatory cytokines in serum of pigs fed CLA 0%, CLA 1% and CLA 2%).

A) IL-1β and **B)** IL-6 on sampling days. Different letters between treatments in the same week indicate significant differences (p <0.05).

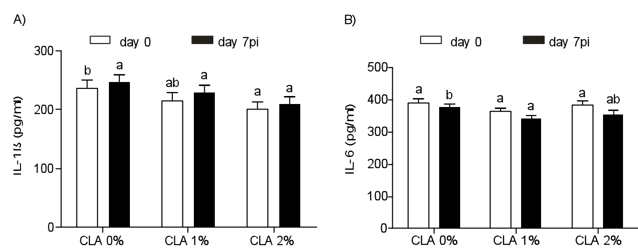
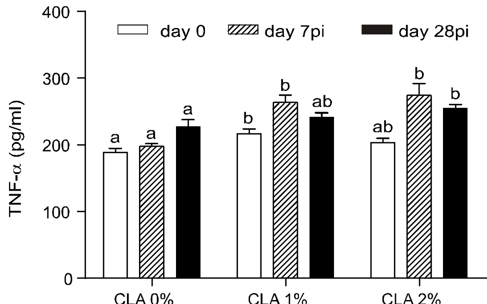


Figure 2. Production of TNF-α in serum of pigs fed CLA 0%, CLA 1% and CLA 2%, on sampling days. Different letters between treatments indicate significant differences (p <0.05).



Discussion

Our results showed that 1% dietary CLA decreased the production of IL-6 in PRRSV infected pigs. In contrast, the production of IL-1β in serum at day 7 pi was not affected by CLA. On the other hand, for TNF-α the pattern of response to CLA differs to the reported in previous works (1,2). However, it is important to emphasize that is the first study involving CLA supplementation in pigs infected with PRRSV, suggesting that the virus could be responsible for the discrepancy in the response found.

Acknowledgements

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AN EVALUATION OF INTERVENTIONS FOR REDUCING THE RISK OF PRRSV INTRODUCTION TO FILTERED FARMS VIA RETROGRADE AIR MOVEMENT (BACKDRAFTING) THROUGH IDLE FANS

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Introduction

The economic impact of porcine reproductive and respiratory syndrome virus (PRRSV) has been recognized worldwide (1). Airborne transmission of the virus is an important route of spread of PRRSV between farms (2). As a means to reduce this risk, Pitkin and others demonstrated the ability of air filtration to prevent the introduction of PRRSV-contaminated bioaerosols to susceptible populations (3). Based on these data, several North American production systems have implemented air filtration systems (4). While preliminary results are promising, a major risk that currently exists in filtered herds under negative pressure ventilation is the retrograde movement of PRRSV-contaminated bioaerosols through non-filtered points i.e., inactive fans (5). To reduce this risk, several interventions have been developed but not validated. Therefore, the objectives of this study are to demonstrate that the risk of back-drafting of PRRSV-contaminated aerosols is a true risk and to validate commercially available interventions.

Materials and methods

The study was conducted at the UMN SDEC production region model (3). Using an existing 25m² facility (void of pigs and ventilated via negative pressure) one of the two 30cm fans was intentionally stopped while the other continued to operate, creating retrograde movement of air into the facility via the inactive fan. This fan, located on the south end of the building was equipped with a standard plastic shutter commonly encountered in commercial swine farms. The operational fan was located at the north end of the facility. All other inlets to the facility were closed resulting in a static pressure of 2.45 Pa.

Besides the standard plastic shutter (A), treatments tested included a plastic shutter plus a canvas cover (B), a nylon windsock (C), an aluminum shutter plus a windsock (D) and a double shutter system involving both an aluminum and plastic shutters (E). All 5 treatments were challenged with 4 different aerosolized concentrations of PRRSV ranging from 1 to 7 logs of virus/L, generated via a cold-fog mister (6) located exterior to the facility 46cm from the inactive fan. To determine whether aerosolized PRRSV could penetrate the treatments, a cyclonic collector was placed inside the facility (3,4) 45cm from the inactive fan. Ten replicates were conducted per treatment, each replicate was 1 minute in length and air samples were tested for the presence of RNA PRRSV by PCR.

Results

Results are summarized in Table 1. Retrograde movement of air in association with the introduction of PRRSV to the interior of the facility was observed during the assessment of treatment A (plastic shutter alone) and B (plastic shutter plus canvas cover). PRRSV introduction to the facility was not observed following the application of the other interventions.

Table 1. Performance of treatments according to challenge dose

PRRSV concentration	A	B	C	D	E
$10^{1}TCDI_{50}/L$	10/10	4/10*	0/10*	0/10*	0/10*
$10^{3}TCDI_{50}/L$	10/10	3/10*	0/10*	0/10*	0/10*
$10^{5}TCDI_{50}/L$	9/10	3/10*	0/10*	0/10*	0/10*
$10^{7}TCDI_{50}/L$	10/10	6/10	0/10*	0/10*	0/10*

A-E: Treatments
*: significantly different when compared to plastic shutter alone ($p<0.05$)

Discussion

Under the conditions of this study, the introduction of PRRSV secondary to the retrograde movement of air was proven to be a true risk which was not eliminated following application of a standard plastic shutter or through the addition of canvas covers. In contrast, interventions such as double shutter systems or shutter plus windsock combinations appear to eliminate this risk. Therefore, a program to minimize the risk of retrograde movement of air into filtered facilities appears to be critical for reducing the airborne risk of PRRSV.

Acknowledgements

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EVALUATION OF SOW LONGEVITY IN PRRSV INFECTED SWINE HERD AFTER THE LONG-TERM USE OF AN INACTIVATED PRRSV VACCINE

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Introduction

PRRS is one of the most significant and economically important infectious diseases in global pig populations (1). PRRSV is a significant cause of production losses in most swine producing countries, inducing reproductive failure in sows, which is mainly characterized by reduction of their fertility and longevity (2). The aim of present study was to evaluate the sow longevity in a PRRSV -infected swine herd after the long-term vaccination with an inactivated PRRSV vaccine, based on an European strain (PROGRESSIS®).

Materials and methods

The trial was performed in a 1000-sow farrow-to-finish pig farm, infected with PRRSV. All gilts and sows were vaccinated with PROGRESSIS®" twice 3-4 weeks apart, except sows or gilts being from 1 week prior to 2 weeks after service, which were vaccinated with a 3-week delay. Furthermore, all sows and gilts received a booster vaccination between 55 and 60 days of pregnancy.

All previously vaccinated animals received a booster vaccination between 55 and 60 days of next gestation, and thereafter at each gestation for a period of 18 months. The replacements gilts after the start of vaccination were vaccinated twice at a 3 - 4 week interval at least 3 weeks before the first service and boostered in each pregnancy as described previously. For each gilt and sow, reproductive data were collected starting from 12 months prior until 18 months after the start of vaccination. Culling rate was calculated. Also, the causes of culling (reproductive failure, death, old age, locomotor problems and other such as injuries, poor body condition, uterine prolapses, urinary tract infections, heat stroke) were recorded during the trial. Data was analysed with one-way Anova (SYSTAT® version 5.0, copyright 1990-1994).

Results

Culling rate of female breeding stock (sows/gilts) per semester and totally prior and after the start of vaccination and the cause of culling rate in the same periods are shown in Table 1. As presented in Table 1 the vaccination lead to significant reduction (p<0,001) of culling rate due to reproductive failure 1.5 years after the start of vaccination Furthermore, culling rate to old age (sows with completion of 8 reproductive cycles) increased (p<0,001) totally 1.5 years after the start of vaccination.

Table 1. Culling rate and causes of culling per semester and totally prior and after the start of vaccinations.

Causes	Semesters relative the start of vaccination				
	Two prior	One prior	One after	Two after	Three after
Culling rate	23.9%^{ab}	25.0%^{a}	22,9%^{ab}	20.8%^{b}	21.1%^{b}
Reproductive failure	40.8%^{a}	40.2%^{a}	31.5%^{b}	25.7%^{bd}	22.2%^{cd}
Deaths	13.5%^{a}	14.0%^{a}	11.2%^{a}	9.7%^{a}	10.3%^{a}
Old age	14.6%^{b}	15.0%^{b}	30.4%^{a}	34,2%^{a}	37.6%^{a}
Locomotor problems	18.8%^{a}	18.2%^{a}	15,8%^{a}	16.5%^{a}	16.7%^{a}
Other	12.3%^{a}	12.6%^{a}	11.2%^{a}	13.9%^{a}	13.2%^{a}
	1 year prior		1.5 year after		
Culling rate	24.5%^{a}		21.9%^{b}		
Reproductive failure	40.5%^{a}		26.6%^{b}		
Deaths	13.7%^{a}		10.4%^{a}		
Old age	14.8%^{a}		33.9%^{b}		
Locomotor problems	12.5%^{a}		12.7%^{a}		
Other	18.5%^{a}		16.3%^{a}		

[a, b] Percentages in a row with different superscripts differ ($p \leq 0.05$).

Discussion

Based on study the vaccination of breeding population with a PRRSV inactivated vaccine can improve the sow longevity in a herd, improving the herd age distribution and the number of non productive sow days. In conclusion, the long-term vaccination of female breeding stock of a PRRSV infected farm tion can lead to decrease of culling rate due to reproductive failure and improvement of the sow longevity. This effect of vaccination has economic interesting for pork producers, especially in closed single-site farrow-to-finish farms.

Acknowledgements

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REGIONAL ELIMINATION OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) has become one of the most important diseases in swine production around the world. PRRS was first diagnosed in the United States in 1987.[1] The disease is manifested by reduced reproductive performance, poor growth, decreased feed efficiency and increased mortality. Recently, the cost of this disease in North America has been estimated at USD $560 million per year in the United States.[2] Veterinarians and producers have been able to eliminate the virus from farms but the risk of re-infection is high. Herds become infected through various routes and aerosol[3] transmission is often suspected. Therefore, a regional elimination approach may increase the odds to succeed in the elimination efforts and also in keeping the herds negative afterwards. In this abstract we describe the efforts being undertaken in the United States to eliminate PRRS virus from regions with emphasis on a region in Minnesota that has made substantial progress.

Materials and Methods

In 2004, local producers in Stevens County a county located in west central Minnesota proposed to bring PRRS virus elimination beyond the herd level and start a regional elimination project. This county has many breeding-stock herds with high biosecurity and health standards. The first step was to contact all producers in the county to obtain their farm location as well as a basic description of the type of pigs being raised. Locations were plotted on a map using ArcGIS 9.3 (ESRI, Redlands, CA). The second step was to obtain producer consent for testing and sharing of PRRS virus results. The third step was to have all herds tested through serology and PCR. The PRRS virus in infected herds was further tested to characterize the strain present in the herd. Each location was classified as positive, negative or unknown based on expert opinion of the local veterinarian or by diagnostic tests such as serology and PCR. Infected breeding herds would start a control program called herd closure[4] to stop transmission within the herd. Sites with growing pigs eliminated the virus through depopulation or strict movement of pigs, personnel and equipment. Quarterly meetings were held to share results, validate information and provide scientific information with regards to the latest PRRS findings.

Results and Discussion

A total of 89 farm locations, one truck cleaning facility, one truck service facility and one hog-buying station were plotted on the map. Of the 89 locations, there are 19 with sows, 8 nursery sites, 40 finishing sites, 2 boar studs, 2 boar stud isolation units, two 4H finishing sites, 13 empty sites and 3 sites that have been abandoned. In 2004 there were 31 locations that were thought to have positive pigs. By 2006, the number of positive locations had decreased to 15 and in 2008 there were four positive locations. Presently, there is only one positive location that is under elimination planning. The project has been expanded to include six surrounding counties. Due to the low density of these additional six surrounding counties and the low prevalence of positive herds, the project has been expanded once again to include northern Minnesota where farm location and testing has already started. To date, a total of 411 locations (farms, buying stations and truck wash sites) are in the project from which 40 are positive, 237 are negative and the remaining locations have an unknown PRRS status.

Due to the Stevens County project success and producer encouragement, six other regional projects have been initiated in the United States through a cooperative USDA funded project. A similar approach has been followed in which local producer and veterinarian meetings have been held, farm identification and mapping has been performed followed by testing. These programs are voluntary and producer driven. Through this process every region has been able to identify some of their challenges. Progress has been variable depending on the unique challenges posed within each region. However, producers and local veterinarians' attitudes and intent have not wavered in their intent to regionally eliminate PRRS virus.

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PREVENTION AND CONTROL OF RECURRENT INFECTIONS OF PRRS

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Introduction

Given the ease with which the virus causing Porcine Respiratory and Reproductive Syndrome (PRRS) can reside in the lymph nodes and alveolar macrophages of the lung, it is not surprising that infection can persist in apparently unaffected carrier animals leading to recurrent disease in a herd(1).

Variability in the elimination of the virus from infected animals, and differing individual susceptibilities create further complications in understanding disease outbreaks. The immune response to a pathogenic virus is not always effective against other strains, however similar their genomic homogeneity. Sometimes the response can lead to sterile immunity.

In a breeding herd, it is very important that replacement animals have been effectively immunised to avoid them becoming a source of instability in the herd. This can be achieved either by field infection and/or by vaccination.

Materials and Methods.

From 2005 onwards, an isolated 5,000 sow herd, producing 6kg piglets experienced PRRS outbreaks every year, leading to >30% abortions in some weeks, and an overall abortion level of 12% per quarter. At the end of 2008, a new regime was instituted involving the quarterly vaccination of all sows with Porcilis® PRRS (2) together with the exposure of replacement animals to the same virus as that already present in the herd.

The replacements were 7-8kg piglets raised on separate and isolated farms infected with the same strain (99% homologous) as the production farm. The batches introduced in 2009 and the first half of 2009 were positive to the PRRS ELISA, and the following batches remained negative up to 10 weeks of age when they were injected intramuscularly with $1x10^3$ virus particles.

The pigs were verified as virus positive by PCR one week post injection and negative by PCR prior to being introduced into the herd. In addition, individual seroconversion was confirmed from blood samples taken on the day of injection and one month later. (The kits used were CIVTEST PRRS European Variant, Idexx-X3 and RT–PCR.) The immunisation program was completed by vaccination with Porcilis® PRRS one month prior to being introduced (160 days old).

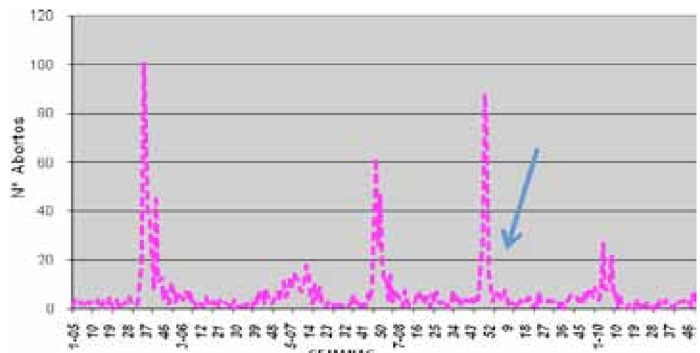
Replacements were not introduced until at least 100 days after the infection, the intakes being batched as far as possible to a few days per month. Although the reception area for new arrivals was the same as that for previous untreated intakes, the sheds were separated from the sows.

Results

All the newly raised batches seroconverted by one month post injection and maintained high antibody titres until they entered the breeding pens. Some groups which had already been seropositive had no positive PCR results, and the newly introduced animals remained negative to PCR.

Reproductive performance improved. Virus circulation was reduced in 2009 and there was no PRRS outbreak in 2010. Recirculation of the virus in the transition of the piglets was not detected until the second half of 2010.

Figure 1. Evolution of abortions per week 2005-2010.



Discussion

The farm remained normally productive throughout 2010, for the first year since 2005. There have been no observations of abortions, returns to oestrus, or failures or delays in conception.

The infection of replacement animals with the strain of virus already in the herd, together with Porcilis® PRRS vaccination, has resulted in their effective immunisation. This regime has avoided the existence of groups of animals susceptible to PRRS at the time they are introduced onto the farm.

The ongoing sow vaccination program is an attempt to maintain the immune status of these animals, and to prevent them losing the level of immunity they had when they arrived as replacements.

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EVALUATION OF SOME SAFETY ASPECTS OF PRRS VACCINATION ON A LARGE POST-SOVIET FARM

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Introduction

PRRSV infection on farms can be controlled by vaccination and farm closure, other management tools, which proved to be quite effective on single-site small farms. However on big multi-site farms PRRS control programs are often failing. Modified live PRRS vaccines are often regarded as posing risk of spreading to unvaccinated pigs, reversion to virulence or recombination of vaccine and field virus circulating in the farm. Field viruses in East Europe are also known to be quite different from the vaccine virus (1, 2).

Aim of this study was to monitor PRRSV circulation on the farm where modified live vaccine was used, to evaluate some safety aspects of vaccination and effect of PRRS control program applied on huge post-soviet pig farm.

Materials and methods

The study was carried out on a 2500 sow farm "B" infected with very diverse EU genotype subtype 2 PRRS virus. Vaccination with EU-genotype MLV Porcilis PRRS was consequently applied for several years, but didn't change the clinical situation. Farm had concerns about homology of the vaccine virus, safety of MLV and overall results so they switched to inactivated vaccines. After this in 2006-2009 for 3 years sampling and analysis of the circulating PRRSV strains was performed. Tissue samples of 2-3 month old piglets were tested in RT-PCR. RNA was extracted from tissue samples using the QIAamp RNeasy kit. Reverse transcription nested PCR specific for ORF5 of the EU-type PRRSV was performed (2, 3). Gel-purified 606 bp ORF5 PCR products were cycle sequenced using the BigDye Terminator Cycle Sequncing kit (v2.0, Applied Biosystems) and the ABI310 genetic analyzer. Sequence alignment was performed using the Clustal W program and phylogenetic tree was constructed with MegAligh software.

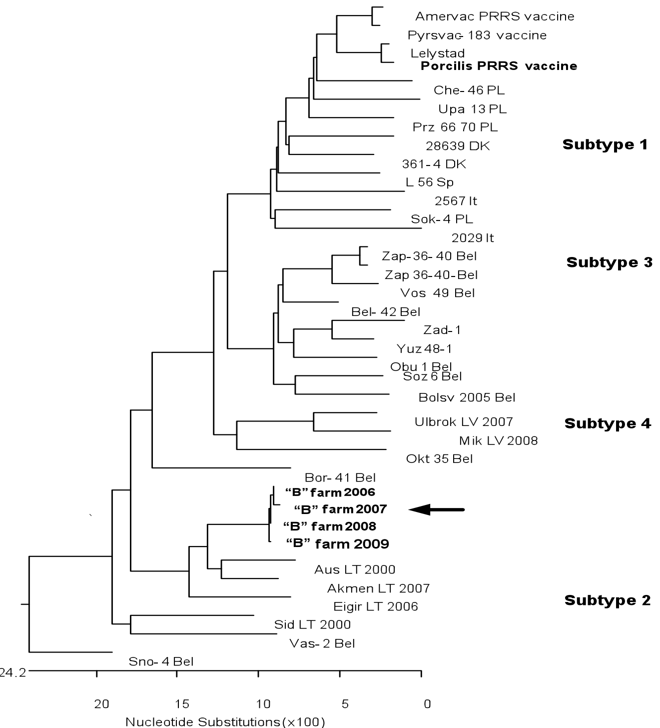
Results

Newly obtained farm "B" PRRSV strains were sequenced in ORF5 and compared to other known sequences from Lithuania, Belarus, Poland and other countries available in the GenBank. Phylogenetic tree was constructed (Figure 1).Vaccine strain of Porcilis PRRS was never detected in farm "B" samples during 4 years monitoring period. Phylogenetic analysis of the detected strains confirmed that only usual subtype 2 field strains were circulating on the farm after vaccination was stopped.

Discussion

We can conclude that the vaccine strain of Porcilis PRRS was not persisting in the unvaccinated pig population after the vaccination was stopped. This is in line with data from trials with sentinel animals stating that spreading of the vaccine strain is very limited (R<1). Therefore Porcilis PRRS can be used safely without risk of spreading and persistence in the unvaccinated pigs. Effect of PRRS control and vaccination program on farm "B" was impaired by wrong pig flow promoting virus circulation and lack of proper acclimatization of gilts. Use of inactivated vaccines also didn't improve PRRS situation and farm had actually returned to Porcilis PRRS vaccination together with changes in pig flow.

Figure 1. Phylogenetic analysis of the persisting PRRSV strains within the large multi-site pig farm during period from 2006 to 2009.



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THE REDUCTION OF PRRS VIRUS INCIDENCE IN WEAK OR STILLBORN PIGLETS BY SOW VACCINATION: ANALYSIS WITH RESPIG

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Introduction and objectives

Pig breeding units often experience problems around farrowing time such as too many piglets born weak or dead due to late abortions and/or early farrowing. The origin of these problems is often complex, i.e. frequently multiple agents are isolated, sows may be in suboptimal body condition, and housing and/or other management factors may be inadequate.

ResPig is a digital diagnostic and monitoring program for veterinarians, involving a biannual cross-sectional serological investigation of respiratory pathogens such as PRRS, PCV2, *A. pleuropneumoniae*, *M. hyopneumoniae*, influenza and *H. parasuis*. The ResPig tool can, if necessary, be expanded by the use of virological investigation and routine necropsy, and it includes an objective scoring system for possible risk factors under the headings of environment, management, housing and biosecurity.

The program helps towards a structured approach to disease prevention. Because of the high number of participating farms and the standard sampling protocols, it is possible to determine the relationship between the infections and technical performance parameters (1). Furthermore, ResPig can be useful in evaluating the efficacy of preventive strategies.

Because PRRS is responsible for reproductive as well as respiratory disorders, ResPig was used in the present study to evaluate PRRS-vaccination in respect to outbreaks of late abortions and/or early farrowing.

Materials and methods

Between 2004 and 2007, PRRS virus isolation was carried out on all weak and stillborn piglets on affected farms with late abortions and/or early farrowing. Sixty-four cases were reported and from these farms weak and/or stillborn piglets were subjected to PRRS virus isolation. In fifty-three (83%) of these cases sows were vaccinated against PRRS: 11 with an inactivated PRRS vaccine, and 42 with a modified live vaccine based on an EU-strain (Porcilis® PRRS) (see Table 1).

Results

Table 1. PRRSv-isolated from weak/stillborn piglets from 64 cases with late abortions and/or early farrowing.

PRRS vaccination sows		PRRS positive	PRRS negative	Total
No		6 (55%) *	5 (45%)	11
Yes		11 (21%) *	42 (79%)	53
	KV	6 (55%)	5 (45%)	11
	EU-MLV	5 (12%)	37 (88%)	42

*OD ratio: 0,2 ; P value: 0,0308

Discussion and conclusion

In most cases of reproductive problems possibly due to PRRS (late abortions and/or early farrowing) PRRSv could not be detected (47 cases negative vs. 17 cases positive). The risk of isolating PRRSv from weak or stillborn piglets was nearly 5 times greater on farms which did not vaccinate than on farms which vaccinated the sows (Table 1). There was no difference between farms using the inactivated PRRS vaccine and farms which did not vaccinate at all in the risk of isolating PRRSv from weak or stillborn piglets.

The PRRS vaccination of sows is likely to be effective in reducing the risk of weak and stillborn piglets due to PRRSv. In this study, the MLV vaccine was more effective than the inactivated vaccine. This has also been found in other studies (2, 3, 4).

ResPig serology, together with disease history, technical and slaughterhouse results, if necessary, complemented by virological investigations and necropsy, make it possible to identify the infections which lead to poor performance. The opportunity to evaluate different preventive strategies helps the veterinary adviser to develop more successful programs, as demonstrated in this study with respect to PRRS sow vaccination.

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VACCINATING PRRS-NAIVE PIGS THAT HAVE TO BE PLACED IN HOG DENSE AREAS

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Introduction

Placing PRRS-naïve pigs into finishing units that are considered at danger of becoming positive can be a challenge, particularly in hog dense areas. This paper shows the results obtained when Canadian PRRS-naïve pigs were vaccinated or not with a commercial PRRS vaccine. A danger score was attributed to the finishing units receiving the pigs and the mortality results in relation to that score were obtained for vaccinated and unvaccinated pigs.

Materials and methods

A danger score was produced based on 4 criteria: previous contamination of the site (1.5, 3.0, 4.5 and 6.0 points for 1, 2, 3 or 4 contaminations); size of the site (0, 0.5, 1.0 or 1.5 points for < 1500, 1500-3500, 3501-6000 or > 6000 pigs); size of the finishing unit (0 or 1.0 point for < 1000 or ≥ 1000 pigs) and proximity of other pigs (any pigs within 1 km, 1.0 point; 0, 1.0, 1.5, 2.0 or 2.5 points for 0, 1-1500, 1501-3500, 3501-6000, or > 6000 pigs within 3 km). Mortality rates in finishing units were compiled for pigs coming from the three same sow herds and that were vaccinated or not with one of the commercial PRRS vaccines (Ingelvac PRRS ATP or Ingelvac PRRS MLV) available in Canada. Pigs were vaccinated in the first 5 days after placement in finishing units.

Results

Table 1 shows the results that were obtained in terms of mortality and mortality per danger score. The results are for pigs raised between 2004 and 2010, so include the period when PCVAD was a problem and no vaccines were available. They are also contemporary, which means that vaccinated and non vaccinated pigs were raised at the same time.

Table 1. Mortality in finishing units for pigs that were vaccinated or not with a commercial PRRS vaccine.

	Vaccinated	Controls	P value
Batches	194	182	
Pigs	252,664	233,805	0.88
Mortality	4.49%	4.81%	0.08
Danger Score	8.00	3.86	<.01
Mortality/DS*	0.56%	1.31%	<.001

*Mortality/danger score

Discussion

It is clear that the risk for PRRS-naïve pigs to become infected with PRRS virus varies according to different criteria. The size of the barn or site and the localtion of that site in relation to neighbouring pigs have been shown to have an impact (1,2,3). The larger the barn or site, the more pigs in the area and the more likely the pigs are to become infected with PRRS virus. Results obtained within the same company in a preliminary evaluation showed that negative pigs placed in hog dense areas were 11 times more likely to have PRRS outbreaks than those placed in sites of low to moderate density(1). In the present study while the mortality rate obtained is only marginally better for vaccinated compared to unvaccinated pigs, it is much better when the risk associated with the different finishing sites is considered. In other words, the mortality/danger score was significantly improved in vaccinated pigs (0.56% vs 1.31%). Furthermore this improvement in mortality/danger score was noted for vaccinated pigs of all three sow herds (0.66% vs 1.84%; 0.66% vs 1.15%; 0.35% vs 0.93%). Performance improvement following vaccination of PRRS-negative pigs placed in hog dense PRRS-positive areas has also been observed by other authors (4).

Recently a licence has been obtained in the US and Canada for mixing of a PCV2, a *Mycoplasma* and a PRRS vaccine. First the PCV2 vaccine is mixed with the *Mycoplasma* vaccine, and then the PRRS vaccine is dissolved in this mixture. Pigs can be vaccinated with a convenient, single 2 mL dose against 3 pathogens. Today more than 100,000 PRRS-naïve pigs have been vaccinated that way within our company. These pigs are now vaccinated in the nursery and the results in terms of safety, efficacy and convenience have led us to pursue that strategy.

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P.157
VACCINATING SOWS AND GILTS FOR PCV2 IN PRRS POSITIVE HERDS - A CASE REPORT

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Introduction

PCV2 infection is widespread and is known to be associated with PMWS, respiratory disease and reproductive failure. Also, it has been shown that PCV2 infection can alter the immune system and thus enhance the susceptibility to secondary infections (1). Coinfections of PRRSV and PCV2 are commonly observed in the field (2). Moreover, piglets from primiparous sows, PCV2 infected sows and farms in an area of high pig density have a higher risk of PRRSV and PCV2 coinfections than piglets from sows of greater parity, non infected sows and farms in low density area (3). This abstract describes the use of PCV2 vaccination in gilts and sows on a 2.350 head sow farm in a pig dense area in Canada and analyses the performance before and after introduction of gilt and sow PCV2 vaccination. Historically the herd had experienced PRRSV contamination with heterologous strains once per year with a resulting negative impact on sow productivity.

Materials and methods

The farrow-to-wean sow farm introduced PCV2 vaccination in piglets (Ingelvac CircoFLEX®) early 2007. Since then, future replacement gilts are also vaccinated on the farm of origin at 6 weeks of age. Gilts are transferred to the acclimatization unit at 55 days of age where they are exposed to the homologous PRRSV strains of the sow farm. Introduction of PCV2 vaccination resulted in a reduction of gilt morbidity and mortality in the acclimatization barn of 50% to 70%. This positive response, the fact that several gilts were serologically negative to PCV2 before entering the sow herd and the finding that a number of gilts became PCV2 positive during the first parity, led the system to start PCV2 vaccination of sows, as well.

Since August 2008 gilts, being vaccinated in the nursery, are vaccinated again at 200 days of age before entering the sow herd. Sows are regularly vaccinated at day 60 of each gestation (Ingelvac CircoFLEX® 1ml IM). Sow productivity data before (September 2006 to October 2008) and after PCV2 vaccination of the sows and 200 day gilts (November 2008 to August 2010) was compared. Data were analyzed using analysis of variance (ANOVA) with the MIXED procedure, (PASW Statistics 18 IBM Corporation Somers, NY).

Results

Improvements in reproductive parameters are summarized in table 1. A significant increase in the farrowing rate, number of pigs born alive and pigs weaned per sow was observed in vaccinated breeding animals.

No effect was observed on the number of mummified or stillborn pigs.

Table 1: Selected reproductive parameters.

	Before - Controls (mean and std error)	After-Vaccinated (mean and std error)	P-value
# periods (months)	26	22	
# sows	12,983	10,648	
Farrow Rate	88.9 (0.53)	90.3 (0.55)	<0.05
Adjusted Farrow Rate	90.2 (0.57)	91.8 (0.50)	<0.05
Born Alive	11.7 (0.05)	12.3 (0.06)	<0.05
Pigs Weaned per Sow	10.2 (0.07)	10.6 (0.06)	<0.001

Discussion

PCV2 sow and gilt vaccination seems to have decreased the impact of PRRSV contamination as shown by the improvement of reproductive parameters. In fact, farrowing rate, pig born alive and weaned per sows has improved even with heterologous PRRSV strain introduction. This indicates that protecting the gilts and the sows against PCV2 might have had a positive effect on the immune system thus reducing the negative impact of PCV2 in a PRRSV and PCV2 coinfected farm. Improvement of reproductive performances could have also been associated with a milder PRRSV strain introduction, heterologous protection or management issue. Production data analysis of similar PCV2 vaccinated sow farm from the same integrated production system, would indicate if similar results are obtained.

In conclusion, this before-and-after comparison indicates that PCV2 vaccination of gilts and sows might have beneficial effect on immune system and on reproductive parameters in a PRRS positive sow herd. Further controlled side-by-side studies might be helpful to better understand the findings of this field observation.

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P.158

SUCCESSFUL CONTROL OF HP PRRS WITH INGELVAC® PRRS MLV IN A 3500-SOW PIG FARM IN NORTH CHINA

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Introduction

Highly Pathogenic Porcine Reproductive and Respiratory Syndrom Virus (HP PRRS) emerged in China in 2006 (1). It broke out again in May 2009 in Jiangsu, Anhui provinces and re-emerged for the third time in May 2010, HP PRRS swept over the major swine rearing areas in China within 4 months. Controlling HP PRRS and reducing its economic impact are priorities for the government and hog producers. One challenge study already showed that Ingelvac® PRRS MLV was effective in reducing the clinical signs and lesions when challenged with an HP PRRS strain (2). This case control report confirms the efficacy of Ingelvac® PRRS MLV against HP PRRS in a the field particularly in a 3500 sows pig farm in Northern China.

Materials and methods

A 3,500 sow farm had HP PRRS outbreak in September 2009. Sows and suckling piglets in farrowing houses, pigs in nursery and fattening houses showed depression, lethargy, high fever (40-42), and thumping. The mortality in suckling pigs ranged form 20% to 40% batch by batch. It was even higher in nursery pigd with a peak of mortality at 68% in January 2010. Almost all nursery pigs showed clinical signs. For fattening pigs and sows, the mortality was nearly 20% and 2% respectively. During this time, a local attenuated PRRSV vaccine was being routinely administered along with various supportive antibiotics. Despite these inputs, production performance was still low. The presence of HP PRRSV was confirmed by RT-PCR test (3).

It was then decided that Ingelvac® PRRS MLV vaccination was to be implemented starting from March, 2010. The vaccination regimen was as follows: sows: whole herd mass vaccination, twice in first month, interval 3 weeks, then 1 time every 3 months; piglets: 2 weeks of age.

A comparison of sow, piglet, nursery and grow-finish mortality 6 months before and 6 months after vaccination was made.

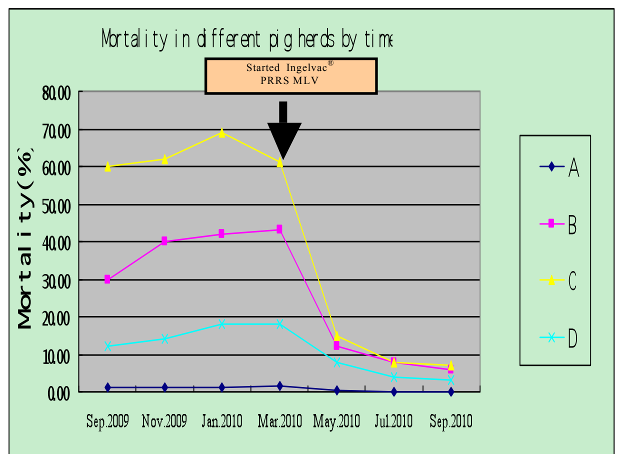
Results

The average mortality of sow herd (A) 1.6% was reduced to 0.4%. For piglets in farrowing house (B) from a mortality of 43% it was down to 12%. Nursery pigs (C) from 61% to 15% . Finally for fattening pigs (D) , from 18% to 8%, all within 2 months post vaccination of Ingelvac® PRRS MLV (Figure 1).

Discussion

This case report has proven that Ingelvac® PRRS MLV was effective in reducing the clinical signs and the mortality caused by HP PRRS virus in field. This is comparable to a previous field control case[4]

Figure1. Mortality of different pig herds in "before-and after" vaccination of Ingelvac® PRRS MLV



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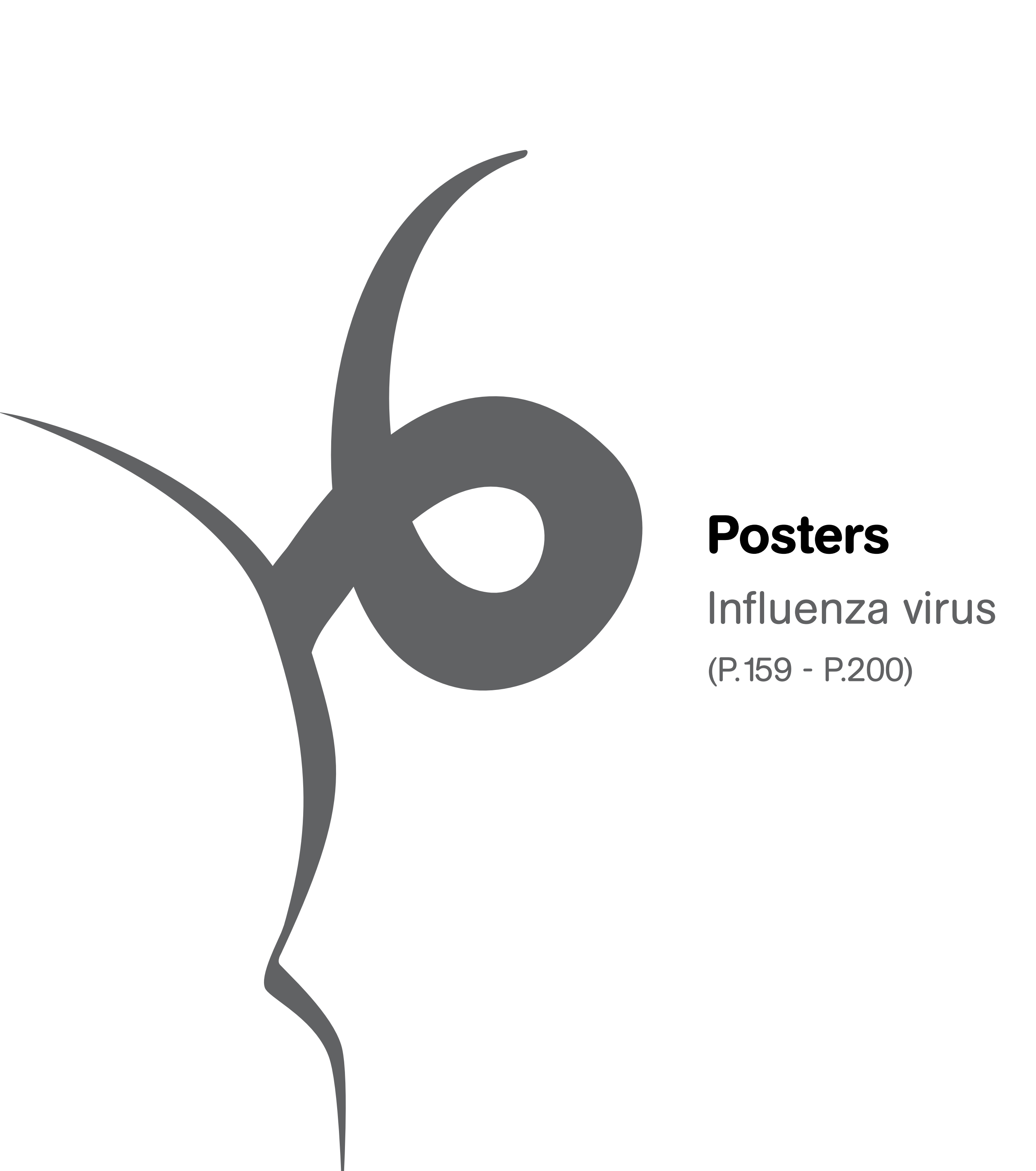

Posters

Influenza virus

(P.159 - P.200)

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PHYLOGENETIC ANALYSIS OF INFLUENZA A VIRUSES DETECTED FROM OUTBREAKS IN SPANISH SWINE HERDS

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Introduction

Swine influenza virus (SIV) infection is most often associated to respiratory disease outbreaks in swine farms; however, it has been shown that SIV seropositive animals can be found in farms where no evident respiratory disease occurs (1). H1N1, H1N2 and H3N2 are the three commonest SIV subtypes isolated in pigs. For H1N1 and H3N2 genetic and antigenic differences can be noticed in isolates of different geographic areas. Those differences arise from the predominance of classical or avian-like lineages and from the antigenic drift of the virus. In addition, for H1N2, human-like and avian-like H1 can be found (2). The objective of the present study was to preliminary assessing SIV subtypes and strains present in outbreaks in Spanish pig farms.

Material and methods

Swine influenza isolates examined in the present study were obtained from nasal swabs or lungs collected from 2008 to 2011 in epidemic outbreaks (n=8). Initially, samples were examined by means of an in-house TaqMan real time one-step RT-PCR and, subsequently, positive samples were inoculated in SPF chicken embrionated eggs. Viral isolation was screened by the hemagglutination test using chicken red blood cells. Isolates were subtyped using specific primers for the partial amplification of H1, H3, N1 and N2 genes as previously described (3). PCR products were sequenced using the Big dye Terminator v3.1 cycle sequencing Kit (Applied Biosystems, Spain). Multiple alignments were carried out by using CLUSTAL W and phylogenetic trees were generated using MEGA 4 software. For comparative purposes, H1 (n=137), H3 (n=62), N1 (n=94) and N2 (n=40) sequences retrieved from GenBank and accounting for swine, avian and human influenza A virus isolates from Europe, Asia and America were used in the phylogenetic analysis.

Results

In all the outbreaks analyzed, the animals affected showed classical signs of swine flu and nine SIV viruses were detected (table 1). In one case, farm nº 4 (F4), two different H1N1 strains were circulating in pigs although co-infection was not detected in the same animal. The two H1N1 strains were 90% similar in the H1 fragment amplified. Phylogenetic analysis showed that H1N1 strains from farms 2, 4 and 6 were close to most of the Euroasian avian-like H1N1 isolates. For F8, the H1N1 virus detected clustered with human A/2009/H1N1 pandemic isolates sharing >97% of similarity with them in the H1. H1N2 and H3N2 strains belonged all to the European SIV cluster.

Table 1. Subtypes of influenza A virus detected in the sampled herds. In bold type, production units were diseased pigs could be found.

Farm	Production units in farm	Subtype	Influenza A cluster
1	**S**	H3N2	European SIV
2	**F**	H1N1	European SIV
3	**S** + W	H3N2	European SIV
4	**F**	H1N1	European SIV
		H1N1	European SIV
5	**S**	H3N2	European SIV
6	**S + W + F**	H1N1	European SIV
7	**S + W** + F	H1N2	European SIV
8	S + W + **F**	H1N1	Human pandemic

S: Sows; W: weaners; F: Fatteners

Discussion

The present work evidences that different SIV strains belonging to the same subtype may-coexist in a farm. A question that remains unsolved is how this co-existence may affect the clinical outcome of the infection as occurred in F4. Even, it could not be discarded the hypothesis of an epidemic H1N1 outbreak in a farm already infected endemically by a different H1N1 strain. Phylogenetic analysis of the H1N1 virus in F8 indicated a relationship with the human 2009 pandemic virus. It is worth to note that this virus was detected in a 2011 outbreak. Taken together, these results suggest that epidemiology of SIV is probably more complex than previously thought and that the potential for viral reassortants to arise in pig farms needs to be carefully re-evaluated.

Acknowledgements

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VIROLOGICAL AND SEROLOGICAL DETECTION OF AN EMERGING VARIANT OF THE H_1N_2 SWINE INFLUENZA VIRUS IN SPAIN

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Introduction

The most prevalent swine influenza virus (SIV) subtypes in Spain are the avian-like swine H1N1, the reassortant human-like swine H3N2, and the reassortant human-like swine H1N2 (1). However, multiple reassortment events, together with antigenic drift, are responsible for numerous swine derivatives (2). In 2010 a new variant of the H1N2 SIV subtype (vH1N2) having an avian-origin hemagglutinin (HA) gene was identified in Spain (3). The aim of this study is to provide information about the presence of this variant at the field level.

Materials and methods

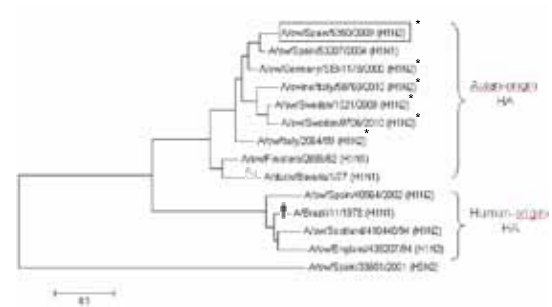
The strain A/sw/spain/5350/2009 vH1N2 was isolated in embrionated chicken eggs in June 2009, from an acute outbreak of pleuropneumonia affecting 3-months-old piglets in a herd located in Southern Spain. Partial HA nucleotide sequence (450 nt) of the vH1N2, along with publicly available SIV sequences (OpenFluDB) were compared (BLAST, CLUSTALW2), and a phylogenetic tree was constructed (neighbor-joining algorithm) with Mega 5. The panel of field samples analyzed included 160 sera (10 sera from 16 fattening batches) used in previous hemagglutination inhibition (HI) experimental studies (Spain 2006-2008): a) SIV-negative (n=60); b) SIV-positive to avian-like swine H1N1 only (n=50); and c) SIV-positive to reassortant human-like swine H1N2 only (n=50). Field sera and the vH1N2 isolate were tested in cross-HI assays, following the European Surveillance Network for Influenza in Pigs (ESNIP) protocol.

Results

The most significant result from the genetic analysis is that HA nucleotide sequence from vH1N2 showed higher similarity (88 to 94%) to the avian-like swine H1N1 SIVs, than to the reassortant human-like swine H1N2 SIVs (61-67%). In fact, vH1N2 clusters together with the avian-like swine H1N1 and other H1N2 SIVs described in Europe, also possessing an avian-derived HA (1,4). (Figure 1).

Table 1 summarizes results of HI assays: A large proportion (60-92%) of field sera in each category reacted strongly (higher GMTs) with vH1N2. Within the group of negative samples, most sera reacted with vH1N2 alone. Instead, samples in the H1N1 and H1N2 categories tested positive also to vH1N2, always showing higher Geometric Mean Titer (GMT) values.

Figure 1: Phylogenetic analysis of partial HA nucleotide sequences of swine, avian and human field and reference influenza viruses.



* Variant H1N2 SIV previously described having an avian-origin HA.
** Sequence generated in this study.

Table 1: Proportion of positive results in the HI assays with selected swine sera (n=160) tested against H1N1, H1N2 and vH1N2 SIVs.

Category of Sera	Test Antigens Proportion of HI positive (≥20) (%) Geometric Mean Titer [GMT]		
	H_1N_1 [a]	H_1N_2 [b]	H_1N_2 [c]
Negative (n=60)	8,3 [23]	0	60 [654,3]
Positive H_1N_1 (n=50)	88 [168]	0	92 [288,6]
Positive H_1N_2 (n=50)	0	96 [169,1]	60 [281,4]

[a]A/sw/spain/53207/2004 (H1N1) avian-like swine,
[b]A/sw/spain/40564/2002 (H1N2) reassortant human-like swine,
[c]A/sw/spain/5350/2009 (vH1N2).

Discussion

Results of this study corroborates that a new lineage of H1N2 SIV, with an avian-origin HA, is currently circulating in pigs in Spain. The unusual serological properties of vH1N2 highlight the limitations of the HI assay as a method to type the SIVs circulating in the field. Furthermore, with the frequent emergence of new variants, the role of molecular methods to help in the surveillance of swine influenza may become crucial. Ongoing work is focussed on sequencing other genes from vH1N2 to understand the origin of the strain. Also the generation of homologous antiserum could help to elucidate divergences in the HI assay.

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P.161

COMPARATIVE HISTOPATHOLOGICAL STUDIES OF FIELD CASES OF H1N1 PDM AND PANDEMIC REASSORTANTS ISOLATES IN ARGENTINA

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Introduction

Pigs are fully susceptible to H1N1pdm as it was reported in several countries including Argentina (1). However, changes such as hyaline membranes, capillary thrombosis and alveolar necrosis observed in field cases were not commonly reported in SIV infection (2). Currently two reassortants from H1N1pdm emerged in pig herds in Argentina (3). The isolates referred as BsAs/H1N1 and StaFe/H1N2 (3) had internal genes similar to the TRIG cassette found in H1N1pdm viruses and HA and NA genes from the human-like H1 and N2 SIVs. Phenotypic and genotypic changes are related with differences in the virus pathogenicity and pathology. This work was aimed to compare statistically the lung lesions found in H1N1pdm and BsAs/H1N1 and StaFe/H1N2 influenza viruses in naturally infected pigs in Argentina.

Materials and methods

A total of 25 lung samples were examined. Nine belonged from H1N1pdm, 8 from BsAS/H1N1 and 8 from StaFe/H1N2. Only IHC and RT-PCR positive samples were analysed. Pneumonia was classified following the morphologic pattern and according the nature of exudates: fibrinous or suppurative. Besides, lesions were scored from 0 to 3 according with the severity observed at level of bronchi, bronchiole, alveoli, connective tissue and pleura. For statistical analysis, ANOVA test and post hoc Fisher's LSD analysis were applied.

Results

The hallmark lesion was a severe necrotizing bronchiolitis. Small and medium sized bronchioles were plugged with neutrophils, necrotic epithelial cells and mucous exudates. Affected airways were denuded or lined by flat epithelium. Bronchi showed degeneration and necrosis of epithelial cells and bronchial gland. Alveolar damage consisted in congestion and thrombosis of the alveolar capillaries, necrosis of the alveolar wall, interstitial and alveolar edema, hyaline membranes, and presence into the alveoli of neutrophils and macrophages. Statistically significant differences were detected among virus isolates ($p<0.0000$). Lesions observed in H1N1pdm were more severe than those found in the new reassortants. When histological structures were disclosed in H1N1pdm bronchiolar lesions were statistically more severe than those observed in others ($p<0.0000$). Bronchial and alveolar lesions caused by H1N1pdm and BsAs/H1N1 were statistically more severe than StaFe/H1N2 ($p<0.0000$ and $p<0.0032$ respectively). Predominant exudative pattern were: fibrinous-suppurative bronchopneumonia (BPn) in H1N1pdm (5/9); fibrinous-suppurative or suppurative BPn in BsAs/H1N (4/8 and 3/8 respectively) and suppurative BPn in StaFe/H1N2 (8/8).

Discussion

The spectrum of lung lesions in pigs appears to vary little among influenza subtypes (4). The work showed that lung changes were more severe in H1N1pdm and BsAs/H1N1pdm than in StaFe/H1N2 particularly at the level of bronchiole. The marked exudative BPn seen in H1N1pdm and BsAs/H1N1 infection is indicative of diffuse alveolar damage. In human beings, extensive areas of alveolar damage were seen after high-virulence influenza virus infection (5). The suppurative pattern seen in StaFe/H1N2 appeared to be of bacterial origin (5) or the results of high level of cytokines secreted by the infected bronchiolar epithelial cells and macrophages which in turn is related with the virus load on these cells (4). Experimental studies are needed to evaluate the consistence of the above described lesions of the reassortant isolates.

Acknowledgements

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P.162
DETECTION OF A HORSE-DERIVED H3N8 INFLUENZA VIRUS IN PIGS IN BRAZIL

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Introduction

Swine influenza virus (SIV) is a commonly isolated pathogen of the porcine respiratory disease system. SIV causes an acute, infectious, respiratory disease with the occurrence of outbreaks in non immune herds. The infection has worldwide distribution in pig producing countries and is caused by various SIV subtypes with genetic makeup from multiple origins, including avian and human viruses (1). Although the disease is well characterized in many countries, little is still known in Brazil of the infection of pig herds with SIV.

The objective of the present work was to characterize a novel H3N8 SIV strain isolated from pig lungs in Brazil, in 2008.

Materials and methods

A total of 70 lung tissue samples from finishing pigs showing pulmonary lesions resembling SIV infection were collected at slaughter, from 2 herds located in Toledo city, Parana State, southern Brazil. Viral isolation was carried out in SPF embryonated chicken eggs. Viral detection was done by RT-PCR and sequencing. Primers for sequencing the M and NA genes were retrieved from Chan et al. (2). Primers for the HA gene were provided by the Avian Influenza Reference Laboratory, NVSL, APHIS, USDA. The sequencing reactions employed BigDye Terminator chemistry and the products were run on an Applied Biosystems 3130xl Genetic analyzer. Consensus sequence was generated using the SeqScape v2.5 software (Applied Biosystems). NCBI BLAST analysis was conducted to identify related reference viruses available in GenBank. A phylogenetic tree of HA gene segment was constructed using the neighbor-joining method in the MEGA 5.01 software based on nucleotide sequences.

Results

Five out of seventy (7.1%) samples were positive for influenza A by RT-PCR. Two samples were isolated in chicken eggs, confirmed by the HA test and the amplification of the influenza M gene by RT-PCR (3). Partial CDS of HA (727bp), M (938bp) and NA (960bp) were subjected to Blast analysis. The Brazilian SIV isolates were closely related (Table 1) to an American H3N8 equine influenza virus (EIV). Phylogenetic analysis (Mega 5.01 software, Neighbor joining, number differences model with 500 bootstraps) of the HA gene (nt918-1604) of the H3N8 SIV isolates with GenBank sequences from equine, avian and swine viruses from the Americas and Eurasia indicated that the Brazilian swine H3N8 virus grouped with a prototype H3N8 EIV, A/eq/Miami/1/1963/H3N8 (CY028836) and the only other available H3N8 sequences of equine viruses from Brazil (A/eq/SP/6/1963, CY032293; A/eq/SP/1/1969, CY032397) and Uruguay (A/eq/Uruguay/1/1963, M24718). The Brazilian SIV isolates did not clustered with avian or avian-derived H3N8 EIV, neither with H3N2 SIVs. The only other reported horse-derived H3N8 SIV isolated from pigs in China (4) is more closely related with later European H3N8 EIV from the 1990s.

Table 1. Blast analysis of Brazilian H3N8 isolates

Gene	Ident. (%)	E - value	Virus designation	Access No
HA	98.49	0.0	A/eq/Miami/1/1963	CY028836
M	100.0	0.0	A/eq/Miami/1/1963	CY028837
NA	100.0	0.0	A/equ/Swit./2225/1979	CY033491

Discussion

The present study showed the first isolation of a horse-derived H3N8 influenza virus in pigs in Brazil. Phylogenetic analyses indicated that the HA gene of the Brazilian H3N8 SIV isolates were more similar to the old American H3N8 EIV than to European H3N8 EIV. In Brazil, serologic studies in the last years detected antibodies against influenza subtype H3N8 in horses. However, the only described case of influenza infection of horses was from two outbreaks of EIV, in Sao Paulo in 1963 and in Sao Paulo and Rio de Janeiro in 1969 (5). Further virological studies in pig populations must to be performed in order to verify if the present case is a sporadic isolation or if H3N8 influenza viruses are established in pigs. Sequencing analysis of all eight viral segments from the Brazilian H3N8 SIV should provide a better understanding of possible epidemiology and origins of these viruses.

Acknowledgements

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ISOLATION AND CHARACTERIZATION OF PANDEMIC H1N1 INFLUENZA VIRUSES FROM PIGS IN BRAZIL

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Introduction

Since the beginning of the influenza pandemic in humans, in April 2009, there were concerns about the possibility of the spillover of this novel influenza virus to swine populations worldwide. Several experimental studies demonstrated the susceptibility of pigs to the pandemic A/H1N1, providing a fast and efficient spread among pigs (1). Consequently, 2 years after the emergence of A/H1N1 in humans, 22 countries have notified to the OIE the presence of Pandemic A/H1N1 in pig herds. In Brazil, until recently, influenza infection in pigs was not considered a problem. Nevertheless, serologic studies conducted in recent years indicated the presence of antibodies against classical SIV H1N1 and H3N2 subtypes in pig herds in 10 Brazilian states (2, 3). However, a recent outbreak of respiratory disease in pigs in 2010, have indicated a possible circulation of a novel influenza virus in pigs.

Herein we describe an outbreak of SIV in pigs caused by the Pandemic influenza virus (A/H1N1/2009), in a pig farm maintained by Embrapa Swine and Poultry Research Center, during sampling of pigs as a part of an ongoing research project.

Materials and methods

On 30 January, 2010, a farm consisting of a 175-sow farrowing- nursery operation with 754 animals that ranged from newborn piglets to nursery pigs showed signs of respiratory disease consistent with SIV infection. Nearly 29% of the pigs were affected (5 sows and 213 nursery pigs) showing clinical signs of fever, cough and loss of appetite, which lasted about 10 days. No clinical signs were observed in piglets and no animal had died. Nasal swabs and lung tissue were collected from twelve infected pigs. Viral isolation was carried out in SPF embryonated chicken eggs and in MDCK cells. Lung tissues were processed for histopathologic examination and for immunohistochemical (IHC) analysis. Viral detection was done by IHC, immunocytochemistry test (ICC), RT-PCR and sequencing. The coding region of Hemagglutinin (HA) gene of influenza virus was amplified in a one-step RT-PCR (Qiagen) using a primer set for the pandemic H1N1/ HA gene (WHO, CDC, Atlanta). Primers for sequencing the M and NA genes were retrieved from Chan et al. (4). The sequencing reactions employed BigDye Terminator chemistry and the products were run on an Applied Biosystems 3130xl Genetic analyzer. Consensus sequence was generated using the SeqScape v2.5 software (Applied Biosystems). NCBI BLAST analysis was conducted to identify related references available in GenBank.

Results

All tested samples were positive for influenza A by RT-PCR. One sample was isolated in cells, confirmed by immunocytochemistry test, using as primary antibody an anti-influenza virus nucleoprotein (5) and by the amplification of the M gene by RT-PCR (6). Histopathologic lesions in lungs were characterized by necrotizing bronchiolitis with mild to moderate interstitial pneumonia. IHC analysis was positive for influenza A. The complete CDS of HA (1769bp) and partial M (897bp) and NA (603bp) genes were constructed with Sequence Scape software. Blast analysis showed 99% nucleotide identity of the HA, M and NA genes with the pandemic influenza virus (A/H1N1/2009) that have been circulating in humans.

Table 1. Blast analysis of Brazilian H1N1 isolate

Gene	Ident(%)	E - value	Virus designation	Access No.
HA	99	0.0	(A/Guandg/55/2009/H1N1)	HQ011423
M	99	0.0	(A/Kenya/0026/2009 H1N1)	HQ214452
NA	99	0.0	A/Guang/45/2009/H1N1)	HQ011420

Discussion

The present study described the first isolation of the Pandemic H1N1 influenza virus in Brazilian pigs. Even though previous serologic studies have indicated the circulation of SIV in Brazilian pigs, little is known about the genetic composition of SIV isolates. In Brazil, pig herds are not vaccinated against SIV, nor is there monitoring of SIV in this specie. Blast analysis showed that the Brazilian H1N1 SIV is closely related with pandemic influenza A (H1N1) viruses that have been circulating in humans.

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FIRST FIELD ISOLATION OF H1N1 INFLUENZA VIRUS AND PHENOTIPIC CHARACTERIZATION IN COLOMBIA, SUDAMERICA

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Introduction

Swine respiratory diseases are a major cause of economic loss in the pig industry of Colombia. In spite of serologic evidence suggesting influenza virus activity since 1970, there is no information available related to the role of this virus on the respiratory disease complex or virus isolation of field strains in Colombia. Nevertheless serological studies in 2003 reported a prevalence of 10% for H3N2 and 0,4% for H1N1 in the three major swine producing regions (1). The main goal of this research is to study serologic activity to swine influenza virus and to characterize strains circulating on the field.

Materials and methods

In order to achieve this, a statistically representative number of 78 herds was selected from the three major swine rearing areas in Colombia. 43 animals per farm were used. The range of age covered animals from nursing, growing to finishing period. A total of 3354 serum samples were tested by indirect ELISA test using a commercial kit (IDEXX) (3) and the HI test using the A/SW/Iowa/1930/H1N1 and the A/SW/Texas/4199/98/H3N2 reference strains (2). Virus isolation was attempted from a total of 275 samples, distributed as follows: 242 Nasal swabs, 25 lung tissue and 8 bronchial aspirates from animals with respiratory signs. Time frame for this study went from 2008 – 2010. Samples were collected in BHI media supplemented with 2% antibiotics, filtered through 0.22 μm membrane and inoculated into 10-11 day-old-embryo chicken eggs and in MDCK cells with trypsin added. HA positive samples were tested by RT-PCR amplification of the M, HA, NA and NS genes. The isolates were evaluated by $TCID_{50}$ in MDCK cells and phenotipically characterized by plaque assay test (6). Full length amplification and sequencing (Macrogen®) of the HA and NA genes were achieved. Phylogenetic analysis was done by Neighbor-Join procedure (5).

Results

Overall, serologic reactivity by HI test was 69,01% to the H3N2 and 49,29% to H1N1 influenza virus. The results of this study confirm the activity of Swine Influenza virus (SIV), on the field which is not surprising considering clinical evidence and the impact of respiratory diseases on the pig industry.

In terms of virus, we found 15 field isolates of swine influenza virus belonging to 9 herds distributed in the three different regions evaluated. 11 isolates were from 2009 (Antioquia n= 6; Occidente n= 5) and 4 isolates from 2010, belonging to the central region.

Table 1. Serologic results by HI Swine Influenza Virus in Colombia 2008-2010.

area	H3N2		H1N1	
	n	%	n	%
Central	4/10	40,0	4/10	40,0
Occidental	19/28	67,9	15/28	53,6
Antioquia	26/33	78,8	16/33	48,5
Overall	**49/71**	**69,0**	**35/71**	**49,3**

Sequence analysis of the HA gene showed that 12 out of 15 isolates corresponded to H1N1 swine origin pandemic influenza virus, and 3 viruses to classic H1N1 influenza virus. Plaque morphology differed between isolates. The classic swine H1N1 virus produced two types of different size plaques while the H1N1 pandemic virus showed uniform medium size plaques.

Discussion

Isolation of the H1N1 swine origin influenza virus for the first time on the field has remarkable impact not only because of its relevance to the pig industry in Colombia but due to the epidemiologic importance and the effect from the public health point of view.

Analysis of the HA gene, showed that the H1N1 pandemic viruses are phylogenetically related with human and swine viruses. The classical swine H1N1 viruses isolated showed homology with virus from United States, Asia and Europe

Acknowledgements

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MOLECULAR CHARACTERIZATION OF H1N1 SWINE INFLUENZA VIRUSES IN ITALY FROM 2007 TO 2010

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Introduction

Swine Influenza virus (SIV) H1N1 subtype was first detected in pigs in Italy in 1976. This H1N1 SIV (classical H1N1) subsequently spread to the rest of Europe, but was rapidly replaced by an avian origin H1N1 virus that had been introduced from birds into pigs. Since the mid 80's the majority of European SIVs are of avian origin (5). Recently the new pandemic H1N1 (2009) has been isolated in respiratory outbreaks in pigs in Italy (6) and other European countries. This new variant was generated as a quadruple reassortant, possessing genes from Euro-Asiatic and American lineages of SIVs, as well as avian and human influenza genes. In this study 19 H1N1 SIVs isolated in Italy from 2007 to 2010, and two SIVs isolates in 2001, were partially sequenced. Haemagglutinin (HA) and Neuraminidase (NA) genes were characterized and compared to sequences retrieved from GenBank or collected in previous studies (2).

Materials and methods

Viruses were isolated from either nasal swabs or lungs collected during outbreaks of respiratory disease in the Northern Italian pig farms by inoculation onto Madin-Darby Canine Kidney cells, CaCo-2 cells and into SPF chicken embryonated eggs. The HA1 part of the HA gene and a region of 514 nucleotides of the N1 gene (1) were amplified and sequenced. Alignments were performed by CustalW, phylograms were constructed by neighbour-joining method using MEGA 3.1 software (4).

Results

HA. It was confirmed that the majority of the Italian SIV strains had an avian-like HA gene clustering with other H1N1 strains isolated in European countries from 1999, and closely related with A/sw/IV/1455/99, an antigenic variant of the reference strain A/sw/Finistere/2899/82. The HA of these recent strains diverged from the strains circulating in Italy in the 80's and 90's which was related to the A/sw/Finistere/2899/82. The sporadic isolation of H1N1 with human-like HA gene was reported in 2001 with the isolation of two viruses (A/sw/It/5433/2001 and A/sw/It/7704/2001) and in 2009 with one virus (A/sw/It/284997/2009). Moreover the introduction of a classical swine HA of American lineage (A/sw/It/290271/2009 H1N1 pandemic) was reported.

NA. As has been observed for the HA genes, the NA genes of the recent strains clustered with the contemporary European H1N1 SIV, diverging from the old (80's and 90's) strains. Moreover the recent NA genes were more related to A/sw/Finistere/2899/82 than A/sw/IV/1455/99. The two reassortant viruses, with human-like HA, isolated in 2001, had a NA gene closely related to A/sw/IV/1455/99, while the reassortant virus isolated in 2009 possessed a NA gene similar to the contemporary circulating strains. The strain A/sw/It/290271/2009 grouped with the third cluster of pandemic H1N1.

Discussion

The current and predominant SIV H1N1 subtype is the avian-like one. Even if the sporadic presence of reassortment events is reported, reassortant strains, human-like H1N1 and the pandemic strains, didn't emerge as a stable lineage. This observation can be explained by the fact that host immune pressure is not marked in pigs because of the continual availability of young pigs without protective immunity. Reassortant SIV strains didn't seem to be competitive compared to the well established SIV viruses circulating among the susceptible pigs. The Italian H1N1 strains isolated in 2007-2010 showed a progressive evolution similar to other H1N1 SIVs isolated in Europe in the same period and the recent Italian isolates clustered separately from the strains circulating till 1998 in Italy. These observations find confirmation in serological studies performed on convalescent pig sera using reference and field SIV strains (3).

Acknowledgements

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A HIGHLY CONSERVED H1-DERIVED SYNTHETIC PEPTIDE ELICITS CROSS-REACTIVE IMMUNE RESPONSES AGAINST THE PANDEMIC H1N1 VIRUS

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Introduction

Outbreaks involving either H5N1 or H1N1 influenza viruses have posted an increasing threat of potential pandemics. Pigs play an important role for influenza spreading, as reflected with the last H1N1 human pandemia, acting as *mixing vessels* and allowing the generation of new recombinant viruses.

We drove our efforts to identify potential vaccine candidates with broadly protective capability against influenza. Several peptides from the hemagglutinin subunit 1 protein (HA1) were selected on the basis of an informational spectrum analysis and using sequences from the GenBank database (1). The objective of this study was to evaluate the immune response induced in pigs by these peptides and to test their protective effect against a heterologous pandemic human H1N1 strain (pH1N1).

Materials and Methods

Eight conventional cross-bred pigs (8-week-old) were divided into two groups and were intramuscularly injected three times, two weeks apart, with either saline solution or a mix of HA1-peptides containing one H1 peptide and three H5 peptides (15 µg per dose). The three doses were prepared with Freund´s adjuvant: complete, incomplete and without adjuvant, respectively. One month after the last immunization, pigs were intranasally inoculated with 10^6 tissue culture infectious dose 50% ($TCID_{50}$) of the pH1N1, A/Catalonia/63/2009 strain.

Serum and peripheral blood mononuclear cells (PBMC) taken before each immunization, before challenge and at necropsy day (6 days post-infection) were used to detect specific humoral and cellular responses. Specific antibodies were detected using a peptide-based ELISA, by a hemagglutination inhibition (HI) assay and by a seroneutralization test (SNT). Cellular responses were determined by IFNγ-ELISPOT after overnight stimulation with either HA1-peptides or with inactivated pH1N1 virus. Viral loads were quantified by a real-time RT-PCR (RT-qPCR) in bronchoalveolar lavage (BAL) obtained at necropsy.

Results

The selected HA1-peptides induced high titres of peptide-specific antibodies when measured by ELISA (lines in *figure 1*) and they showed low albeit detectable seroneutralizing and hemagglutination inhibitory activities. Interestingly, IFNγ-secreting T-cells were also detected by ELISPOT assay after *in vitro* stimulation with either the specific peptides (bars in *figure 1*) or the inactivated pH1N1. Partial protection was achieved in vaccinated pigs, showing lower viral loads in BAL compared with the control pigs at day six post-infection (*figure 2*).

Figure 1. HA1-peptides elicited both humoral and cellular immune responses when tested by peptide specific-ELISA (lines) and IFNγ-ELISPOT (bars). Average values per time and group are represented.

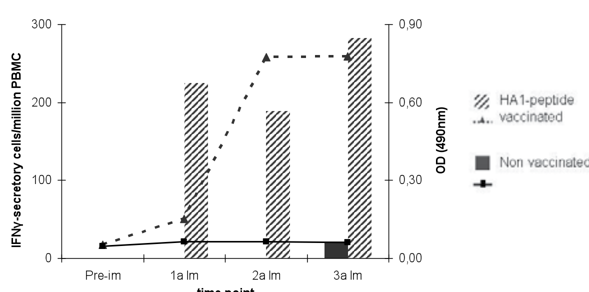
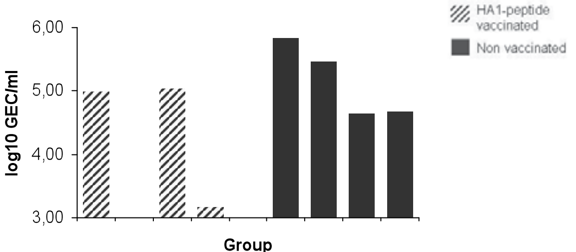


Figure 2. Influenza RNA viral quantitation in BAL 6 dpi. Average values per time and group are represented. The detection limit in the assay is 3 log_{10} GEC/ml.



Discussion

Finding a universal effective vaccine to fight against future influenza outbreaks has become a priority for the scientific community. Despite the peptide-based experimental vaccine here presented was tested in conventional pigs to mimic field conditions, it elicited a complete immune response in all vaccinated-animals. The fact that B and T-cells induced by the vaccine were able to recognize and partially protect pigs against the challenge with the heterologous pH1N1 virus, opens new expectatives for future developments.

Acknowledgements

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MOLECULAR CHARACTERIZATION OF INFLUENZA A VIRUSES FROM SWINE FOR POTENTIAL VIRULENCE MARKERS

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Introduction

As influenza A viruses continue to emerge and evolve through reassortment and antigenic drift in the US swine population, novel strains have arisen over the last decade that have resulted in changes in influenza epidemiology and persistence within endemically infected populations of pigs. The hemagglutinin (HA) gene of all influenza viruses that were detected in samples submitted from one endemically infected farm system (farm M) have been sequenced since 2005. In 2008, a two amino acid (AA) insertion (Glu-Lys) was found at position 156 using the H1 numbering system (1). The viruses with the insertion were isolated from pigs in specific barns in a multi-site rearing system from farm M that were experiencing increases in piglet mortality.

To better understand how this insertion relates to the pathogenicity of these influenza viruses, five viruses with the insertion and three potential ancestral viruses without the insertion were selected for further molecular analysis.

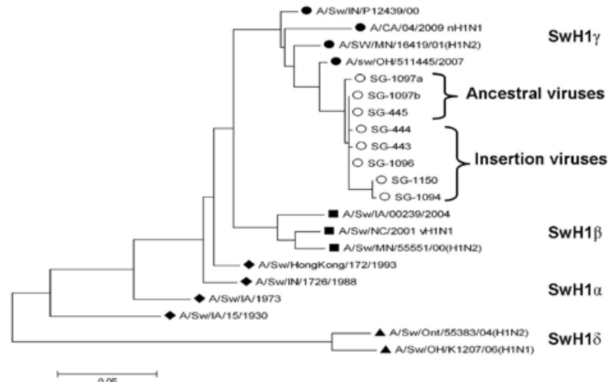
Materials and methods

The eight selected influenza A viruses were isolated on Madin-Darby Canine Kidney (MDCK) cells from swine tissue and nasal swab samples submitted to the University of Minnesota Veterinary Diagnostic Laboratory from farm M as previously described (3). The full genomes were sequenced de novo using Next-Generation Illumina ® sequencing methods at St. Jude Children's Research Hospital in Memphis, TN. The sequences were analyzed using MEGA 4 with Clustal W alignment. The three-dimensional models of the antigenic sites were examined using PyMOL.

Results

Phylogenetic analysis of the viruses revealed that the isolates clustered tightly together with the highly pathogenic virus A/Sw/OH/51145/2007 (H1N1) in the SwH1 gamma clade (2,5; Figure 1). Analysis of the protein sequence revealed that the two AA insertion at position 156 disrupted the Sb and Sa antigenic sites which are proximal to the HA receptor binding pocket in the three-dimensional protein structure (1,4). This was the only variation in the antigenic sites for two of the five viruses when compared to the ancestral viruses. Virus SG-444 also had one AA change at position 70. Viruses SG-1094 and SG-1150 also had AA changes at 74, 139, 162 and 168. The analysis of the other seven genes for all eight viruses revealed that the Neuraminidase (NA) gene had 98.9% to 100% similarity, resulting in 0-8 AA differences in the protein sequences. There was minimal to no genetic divergence detected in the six internal genes for the eight viruses which had nucleotide similarities 99.3-100% and 0-5 AA changes.

Figure 1. Phylogenetic tree of HA gene sequences for the eight viruses (O) analyzed from farm M.



Discussion

Molecular characterization of influenza viruses has become one of the key tools used by researchers to examine the continual changes that are found in viruses isolated from the U.S. swine population. By sequencing the viruses that are endemic in pigs, we can screen the circulating viruses for significant genetic changes and compare multiple viruses isolated from the same population over time to each other, as well as to viruses in available vaccines. These changes will provide a benchmark for evaluating how the virus evolves in swine. This information is critical for understanding the epidemiology of the disease caused by the virus within a farm or production system, especially when the molecular data can be combined with clinical signs and post-mortem findings.

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IN VITRO ASSESSMENT OF INFLUENZA A VIRUS ATTACHMENT IN THE UPPER AND LOWER RESPIRATORY TRACTS OF PIGS

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Introduction

As influenza A viruses continue to emerge and evolve through reassortment and antigenic drift in the North American swine population, strains have arisen that are associated with increased virulence and changes in influenza epidemiology. Over the last decade, many of these new viruses have been evaluated through the use live animal pathogenesis models (1-3). However, there is still a lack of knowledge regarding viral binding patterns of influenza viruses in pigs.

To further characterize and understand the process of infection with different influenza viruses, we need to examine the pattern of virus attachment. Using a technique called virus histochemistry, we can create a new tool for assessing the pathogenicity and phenotypic characteristics of swine influenza viruses in an animal-free model. This technique has been used to demonstrate the relationship between binding patterns and severity of pneumonia in humans (4), but has yet to be applied to swine influenza viruses and respiratory tissues of pig origin.

The objective of this study was to examine the binding patterns of influenza A viruses from swine that have been definitively characterized by prior genetic and antigenic analyses.

Materials and methods

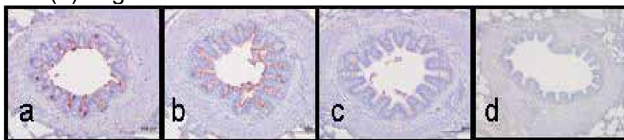
Selected viruses represented the four genetic clusters (α, β, γ and δ) of North American swine H1 (SwH1) viruses (1-3). Additionally, a group of viruses containing a two amino acid insertion in the binding site of the hemagglutinin gene and their presumed ancestral viruses were selected. A/CA/04/2009 H1N1 and a vaccine virus were used as positive controls and the label, fluorescein isothiocyanate (FITC), was used as a negative control.

Virus histochemistry was performed on formalin-fixed paraffin-embedded respiratory tissues collected from healthy pigs that were 6 weeks and 13 months old. FITC labeled influenza viruses (50 to 100 hemagglutinating units) were prepared as previously described (4,5) and incubated on the deparaffinized and rehydrated tissues overnight at 4°C. Using anti-FITC antibody and a tyramide signal amplification system, the signal of the virus label (FITC) was detected and amplified. This signal was revealed with 3-amino-9-ethyl-carbazole which produced the granular red staining on the apical surface of epithelial cells where the virus had attached. The viral attachment to epithelial cells was scored as follows: (–) no attachment; (+) rare or few cells; (++) moderate number of cells; (+++) abundant or many cells.

Results

The virus histochemistry scores per respiratory zone (nasal turbinate, trachea, bronchus, bronchiole, and alveoli) ranged from + to +++ for all viruses. Bronchioles were consistently highest for respiratory binding scores (++ to +++) for all viruses, regardless of animal age. There was no difference in binding between the upper and lower respiratory tract of the 6-week-old pig for the SwH1 viruses characterized in previous studies as highly virulent. Both positive control viruses had mild to moderate binding (+ to ++) throughout the respiratory tract. The negative control had no binding throughout the respiratory tract.

Figure 1. Photomicrographs of bronchiolar viral attachment with (a) ancestral virus (b) insertion virus (c) positive control and (d) negative control.



Discussion

The present study provides new insights on the binding patterns of influenza A viruses from swine to porcine respiratory epithelial cells. Computerized morphometric analyses may be needed to detect subtle differences between viral binding patterns not detected by the naked eye. The information provided in this study combined with the information derived from the genetic analysis of these viruses will provide a benchmark for evaluating future changes in viral binding and aid in the selection of vaccine candidates, as well as aid our understanding of swine influenza virus persistence in pigs.

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SWINE, AVIAN AND HUMAN INFLUENZA A 2009 VIRUS ARE DIFFERENTIALLY SENSED BY CONVENTIONAL PORCINE DENDRITIC CELLS IN VITRO.

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Introduction

Dendritic cells (DC) link innate and adaptive immune system expressing specialized pattern-recognition receptors (PRRs) which recognise particular pathogen-associated molecular patterns (PAMPs). Furthermore, there is growing evidence that the so-called "early" cytokines play an important role in influenza virus infection. It is well known that pigs are susceptible to avian, porcine and human influenza virus and pigs can act as "mixing vessels" for new influenza viruses.

Our main goal was to characterize the differential response of porcine bone marrow derived dendritic cells (poBMDC) or conventional dendritic cells, after infection with swine, avian or human influenza virus *in vitro*.

Materials and methods

Porcine BMDC were generated and DC morphology and virus infection was evaluated by transmission electron microscopy. Porcine BMDC were infected with A/swine/Spain/SF32071/2007(H_3N_2), Highly pathogenic A/chicken/Italy/13474/99(H_7N_1), Low pathogenic A/Anas plathyrhynchos/Spain/1877/2009(H_7N_2), and pandemic A/Catalonia/63/2009(H_1N_1) viruses or stimulated with TLR agonists (Poly:IC, LPS or R837). Additionally, 16h post infection (hpi) with H_3N_2, cells were further stimulated with Poly:IC or LPS for 24h. DC phenotype was analysed by flow cytometry at 24 hours, whereas IFN-α, TNF-α, IL-12 and IL-18 secretion were analysed by ELISA at 4, 8, 16 and 24hpi. IL-10 and TGF-β were analysed by RT-qPCR at 4, 8, 16 and 24hpi.

Results

Infected-poBMDC presented different phenotype by means of SLAI, SLAII and CD80/86 up-regulation. Different cytokine kinetic profile of IFN-α, TNF-α, IL-18 and IL-12 were observed depending on the virus used. Stimulation with TLR agonists induced up-regulation of SLAI, SLAII and CD80/86 and different kinetic profile in secreted cytokines, being high responders to Poly:IC and LPS. Stimulation with Poly:IC o LPS after H_3N_2 influenza virus did not change the profile of secreted cytokines neither the profile of activation markers compared to their controls. No induction of IL-10 and TGF-β mRNA was detected in infected cells.

Discussion

The different responses observed in poBMDC infected with influenza virus or stimulated with TLR agonists pave the way for understanding the interaction between different influenza viruses and porcine dendritic cells and for triggering the mechanisms driving to protective immune response.

Acknowledgements

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PORCINE INFLUENZA VIRUS INTERACTION WITH CONVENTIONAL PORCINE DENDRITIC CELLS

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Introduction

Dendritic cells (DC) link innate and adaptive immune system. Replication cycle of influenza virus has been mainly studied in epithelial cells and the interaction with other porcine immune cells such as conventional DC is largely unknown.

Our main goal was to study the interaction of porcine influenza virus with conventional porcine bone marrow derived dendritic cells (BMDC) *in vitro*.

Materials and methods

Porcine BMDC were generated as previously described (1). Morphology and virus infection evaluated by transmission electron microscopy (TEM). BMDC and Madin-Darby Canine Kidney Cells (MDCK) were infected with A/swine/Spain/SF32071/2007(H_3N_2) at 0.01 MOI for 4, 8, 16 and 24h. After infection, supernatants and cells where collected to evaluate virus progeny by virus titration in MDCK and by RT-PCR respectively. Twenty four hours post infection (hpi) mock or infected cells where collected and treated for conventional and inmunogold staining and analysed by TEM JEOL1440. Furthermore, 24h pi, mock or infected BMDC where collected to perform an infectious centre (ICA) and Transwell (TA) assay using MDCK as permissive cell line.

Results

After infection, an increased virus titre was detected in MDCK compared to BMDC. Using RT-PCR, some replication was detected in BMDC from 4 to 8hpi and then it decreased whereas ΔCT (difference in threshold cycles) increased in MDCK. Using TEM, infected cells exhibited structures resembling influenza virus in the cytoplasm. To establish whether these structures were swine influenza virus, BMDC were stained with anti-influenza nucleoprotein antibody coupled to gold particles. Specific binding was observed inside infected BMDC. Swine influenza virus from BMDC was able to infect other cells in close contact and this infection was dependent on cell to cell interaction.

Discussion

The data presented in this work showed that interaction of influenza virus in no-polarized cells such as DC is different from polarized cells such MDCK. Theoretically, the fact that DC are able to infect cells in close contact make them suitable candidates to spread the virus within the body. The data generated in our studies unravel the particular interaction of swine influenza virus with conventional DC, extending the current understanding of porcine immune response against influenza virus.

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This work was partly funded by the following Projects: CSD 2006-00007, AGL2006-13809-C03-01, AGL2009-12945-C02-01 and AGL2010-22200-C02-01 by the Spanish Government. PhD studies of Mrs. Tufária Mussá and Elisa Crisci are supported by a doctoral grant from the AECID and from the Spanish Ministry of Science and Technology respectively.

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REPRODUCTION OF THE PATHOGENICITY OF PANDEMIC H1N1 VIRUS ISOLATED FROM A KOREAN PIG FARM

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Introduction

Pandemic H1N1 infected pig herds were reported April 28 in Alberta (1), and May in Manitoba (2), Canada in 2009. On May 2, 2009 the first Korean case of pandemic H1N1 was officially confirmed in person who had arrived from Mexico City on April 26, 2009. And pandemic H1N1 occurred in pig farms and the virus was isolated from the pig in 2009 (3). Since the pandemic H1N1 outbreak, there have been several experimental infection studies in pigs using pandemic H1N1 human isolates [4]. The objectives of this study were (i) to determine the genetic identity of a H1N1 virus isolated from a Korean pig farm and (ii) to show if the pathogenicity of the isolate could be experimentally reproduced in specific pathogen free (SPF) pigs.

Materials and methods

Following the monitoring of the pandemic H1N1 after first pandemic H1N1 outbreak in Korean pig farm in December 2009, specimens from the breeding farm with about 6000 pigs were tested for pandemic H1N1 virus using real time RT-PCR specific to pandemic H1N1 (Bionote, Hwasung, Korea) and the results were positive in grower pigs. The virus was successfully isolated only in MDCK cell line after a second passage, and was designated as A/swine/Korea/GCVP-KS01/2009 (H1N1).

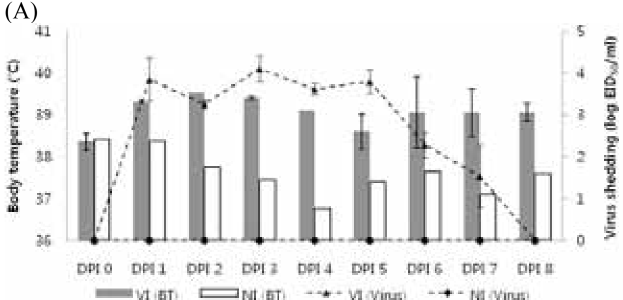
The nucleotide sequence of the isolated pandemic influenza H1N1 was partially sequenced for the all 8 genes.

The virus isolate was nasally inoculated into SPF miniature pigs (Medipig, South Korea). Three 5 week old Sinclair miniature pigs were used in this study. Two of them were used for virus inoculation (VI) group and one pig was used as a non inoculation (NI) control. Two milliliters of virus (2^7HAU of virus, passage level 4) was intranasally inoculated to VI group via intranasal route. Clinical signs including the temperature and the amount of viral shedding in nasal discharge were monitored daily for 8 days post-inoculation (DPI).

Results

The nucleotide sequences of GCVP-KS01 showed 99%, 99.3%, 99.6%, 99.1%, 99.1%, and 99.5% similarity to the A/California/04/2009 strain for HA, NA, M, NS, NP, and PA, respectively. According to phylogenetic analysis for the HA and NA nucleotides, GCVP-KS01 clustered with pandemic H1N1 viruses isolated from human in 2009 to 2010. Interestingly, the HA gene of the pandemic H1N1 virus isolated from humans in Korea was closely related to that of this isolate.

(A)



(B)

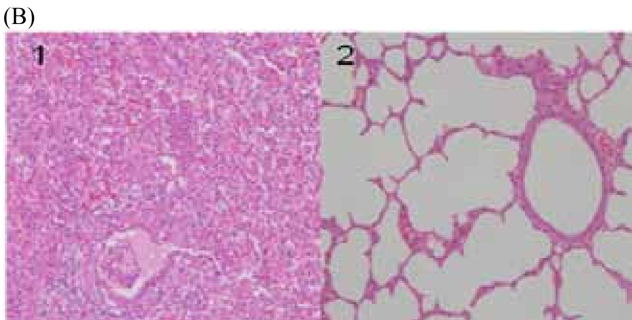


Figure1. (A) Changes of the mean body temperature (BT) and viral shedding (Virus) in the virus inoculation (VI) group and non inoculated (NI) group (B) Lung of a pig infected with an A/swine/GCVP-KS01/2009 (H1N1) virus, showing severe suppurative necrotizing bronchopneumonia (1), or a normal lesion in an uninfected pig (2). H&E stain. Original magnification, x200.

Discussion

As predicted based on previous studies (4), clinical signs were successfully reproduced in the lungs of pandemic H1N1 virus isolated from pig inoculated SPF pigs, including fever. The extended study covering the comparative research with classical swine influenza virus H1N1, H3N2, and H1N2 is ongoing because current study could not suggest the comparative pathogenicity of the pig isolated pandemic H1N1 virus. This study validated the pathogenicity reproduction of pig-isolated pandemic H1N1 virus in SPF pigs, and demonstrates the potential concern of viral circulation of pandemic H1N1 among pig herds.

Acknowledgements

This work was supported by the National Agenda Project grant from Korea Research Council of Fundamental Science & Technology and the KRIBB Initiative program (KGM3111013).

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EXPERIMENTAL INOCULATION OF 5-7 WEEK-OLD PIGS WITH TWO RECENT EUROPEAN ISOLATES OF H1N1 SWINE INFLUENZA VIRUS

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Introduction

The objective of this study was to assess the clinical, pathological and virological outcomes observed in 5-7 week-old pigs after challenge with two recent isolates of swine influenza virus (SIV) H1N1 subtype.

Materials and methods

Viruses: Selected H1N1 strains were Sw/Gent/112/07 and A/Swine/Spain/SF11132C/2007, isolated in 2007 in Belgium and Spain, respectively. Viruses were grown on embryonated chicken eggs until passage level 2 and the titers obtained were $10^{6.8}EID_{50}$/ml (Belgian H1N1) and $10^{7.4}EID_{50}$/ml (Spanish H1N1).

Animals and experimental design: Thirty conventional, SIV-seronegative, 5-7 week-old pigs were stratified by litter and randomly assigned to three different challenge groups (T01, T02 and T03). For challenge, all pigs were anesthetized and intratracheally inoculated with 5 ml of either the Belgian strain (T01), the Spanish strain (T03) or the Spanish strain diluted up to $10^{6.8}EID_{50}$/ml in PBS (T02)(Table 1). Clinical signs and rectal temperatures were recorded the day before challenge and at 0, 6, 24, 30, 48 and 72 hours post inoculation (hpi). Half of the pigs in each group were euthanized at 24 hpi and half at 72 hpi. Lungs were macroscopically evaluated for the presence of SIV-like lesions and tissue samples from both lung halves were collected to perform virus isolation on MDCK cells. The SIV titer was expressed as $TCID_{50}$/g. Groups were not statistically compared because each group was housed in a different room.

This experiment was carried out in compliance with national legislation and subject to local ethical review.

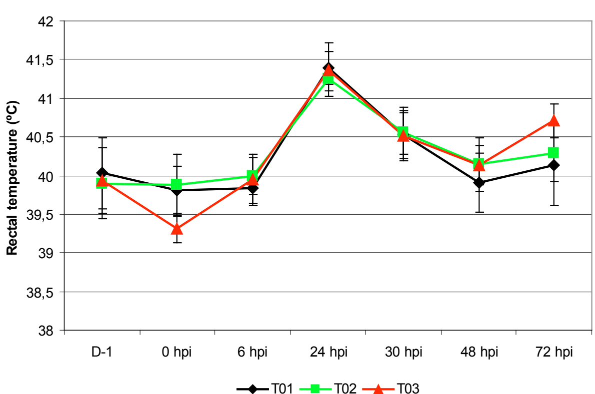
Table 1: Challenge groups included in the study

Group	N	Strain	Virus titer (EID_{50}/ml)	Inoculum dose (EID_{50}/pig)
T01	10	Belgian	$10^{6.8}$	$10^{7.4}$
T02	10	Spanish	$10^{6.8}$	$10^{7.4}$
T03	10		$10^{7.4}$	$10^{8.1}$

Results and discussion

All SIV-inoculated pigs showed elevated rectal temperatures (> 40.5 °C) after challenge (Figure 1). Besides increased rectal temperatures, other typical influenza symptoms including inactivity, labored breathing and coughing were also observed in all three challenged groups. These results are consistent with the clinical outcome that has been previously described following intratracheal inoculation of pigs with high doses of SIV (1).

Figure 1: Pre- and post-challenge rectal temperatures in each challenge group



At necropsy, all pigs but one from the T01 group had macroscopic SIV-like lesions in the lung (Table 2). In addition, results from virus isolation in MDCK cells revealed the presence of virus in both lung halves of all inoculated pigs (Table 2), indicating that the two strains successfully replicated in the lung.

Table 2: Mean values ± SD obtained for lung scoring and virus isolation in each challenged group

Group	Mean lung score[1] ± SD		Mean titer ± SD ($log_{10}TCID_{50}$/g)	
	24 hpi	72 hpi	24 hpi	72 hpi
T01	1.0 ± 0.8	16.3 ± 2.8	7.0 ± 0.3	5.9 ± 0.8
T02	6.0 ± 2.2	24.7 ± 11.8	6.7 ± 0.3	6.6 ± 0.3
T03	5.2 ± 2.5	29.1 ± 6.1	6.3 ± 0.9	6.8 ± 0.5

[1]Percentage of lung consolidation

Conclusions

The results of the present study indicate that the two tested H1N1 SIV strains are able to induce infection and clinical disease in 5-7 week old pigs, when inoculated intratracheally at a dose of ≥ $10^{7.4}$ EID_{50}/pig.

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EXPERIMENTAL INOCULATION OF 6-7 WEEK-OLD PIGS WITH TWO RECENT EUROPEAN ISOLATES OF H1N2 SWINE INFLUENZA VIRUS

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Introduction

The aim of the current study was to evaluate the development of clinical disease and pathology induced by two recent (2007) isolates of swine influenza virus (SIV) subtype H1N2 after intratracheal inoculation of 6-7 week-old pigs.

Materials and methods

Viruses: Sw/Gent/102/07 H1N2 strain was isolated in Belgium and A/Swine/Spain/SF12122C/2007 strain in Spain. Both viruses were passaged two times in allantoic cavities of 10-day-old embryonated chicken eggs to provide virus for the inoculation of pigs. The titers obtained were $10^{5.7}$ EID_{50}/ml for the Belgian strain and $10^{7.4}$ EID_{50}/ml for the Spanish one.

Animals and experimental design: Twenty-nine 6-7 week-old conventional pigs free of antibodies against H1N1, H1N2 and H3N2 subtypes were anesthetized and intratracheally inoculated with 5 mL of Sw/Gent/102/07 strain (T01, N=10), A/Swine/Spain/ SF12122C/2007 strain (T03, N=10) or the Spanish strain diluted in PBS up to the same titer as the Belgian strain (T02, N=9). The monitoring consisted of a clinical assessment of general (depression) and respiratory (abdominal respiration, coughing, sneezing) symptoms and taking the rectal temperature (RT) the day before challenge and at 0, 6, 24, 30, 48 and 72 hours post inoculation (hpi).

Half of the animals from each group was euthanized 24 hpi and the other half 72 hpi. At the time of necropsy, lung gross pathological examinations were performed and samples were collected for virus isolation. Lung homogenates (10% w/v) were prepared in MEME medium with antibiotics, clarified by centrifugation and used for titration in MDCK cells. Statistical analyses were not conducted as each treatment group was allocated in a separate room.

This experiment was carried out in compliance with national legislation and subject to local ethical review.

Table 1: Lung scoring and virus isolation results expressed as mean values ± SD
[1]Percentage of lung consolidation

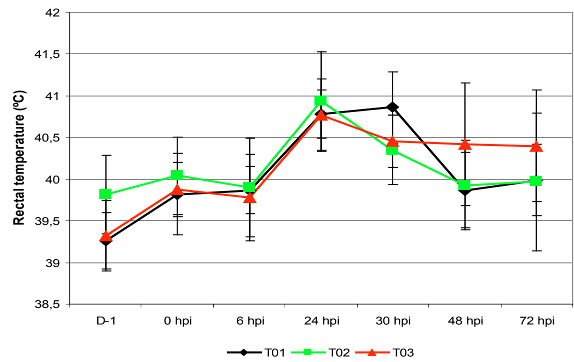
Group	N	Strain	Titer (EID_{50}/ml)	Dose (EID_{50}/pig)	Mean lung score[1] ± SD		Mean titer ± SD ($log_{10}TCID_{50}$/g)	
					24hpi	**72hpi**	**24hpi**	**72hpi**
T01	10	Belgian	$10^{5.7}$	$10^{6.4}$	3.8 ± 1.1	11.3 ± 4.7	6.9 ± 0.6	6.3 ± 0.5
T02	9	Spanish	$10^{5.7}$	$10^{6.4}$	5.3 ± 5.2	16.2 ± 8.7	6.8 ± 0.3	6.2 ± 0.7
T03	10	Spanish	$10^{7.4}$	$10^{8.1}$	2.8 ± 2.6	21.7 ± 5.7	5.6 ± 0.2	6.0 ± 0.5

Results and discussion

In all groups pigs became clinically ill, demonstrating respiratory distress and high rectal temperature (>40.5°C) one day after challenge (Figure 1). In addition, in the group inoculated with the highest titer (T03) temperature remained above 40.5ºC until three days after challenge in 4 out of 5 pigs.

The results are consistent with the clinical outcome that has been previously described following intratracheal inoculation of pigs with high doses of SIV (1).

Figure 1: Pre- and post-challenge rectal temperatures



At post-mortem examination, all pigs revealed macroscopic lung lesions typical of SIV infection. Moreover, virus was isolated in all lung homogenates from the three treatment groups. Results on lung scoring and virus isolation are displayed in Table 1.

Conclusions

Our experimental data demonstrate that both H1N2 strains, Sw/Gent/102/07 and A/Swine/Spain/ SF12122C/2007, are able to develop SIV clinical disease in 6-7 week-old pigs when inoculated intratracheally at a dose of ≥ $10^{6.4}$ EID_{50}/pig.

References

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SEROPREVALENCE OF ANTIBODIES AGAINST SWINE INFLUENZA VIRUS IN FARM SWINES OF DIFFERENT AGE IN CROATIA

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Introduction

Influenza viruses of three different subtypes - H1N1, H3N2 and H1N2- are circulating in pig populations worldwide (1). Influenza virus is a zoonotic agent of concern that could present economic and health challenges to human and animal populations on a global scale (2). In Croatia little information is known about the influenza viruses that exist in swine. The aim of this study was to investigate the seroprevalence of swine influenza H1N1 and H3N2 viruses among Croatian domestic pigs of different age.

Materials and methods

During 2010 a total of 415 sera were collected from non-vaccinated animals of different age. They were raised on 12 farms with high biosecutity practices, located in a density swine populated area in Eastern part of Croatia. Blood samples were collected by jugular vein puncture using tubes without additive. The samples were individually identified, and delivered to the Virology department of the Croatian Veterinary institute in Zagreb. Serum was obtained by centrifugation for 10 min at 2000 rpm and stored at -20 ^{0}C until analysis.

All sera were tested using commercial ELISA (HerdCheck H1N1 and H3N2 Swine Influenza Virus Antibody Test Kits; IDEXX Laboratories, Westbrook, ME, USA) according to the manufacturer's instructions. Samples were considered to be positive for SIV antibody if the S/P ratio was 0.4 or greater.

Results

Regarding all investigated sera (n=415) (Table 1) the percentage of animals with antibodies against H1N1 virus strain was higher (14.2%) than that of animals with antibodies against H3N2 virus (6.5%). Our serological results also show differences between age classes. The highest prevalence was detected among the group of sows and gilts (9.3-50.0%) compared to the weaned piglets and fatteners (2.5-8.6%).

Table1. Presence of antibodies against H1N1 and H3N2 in different age groups.

Group	Number of sera tested	Number (%) of positive sera	
		H1N1	H3N2
Sows	70	35 (50.0)	16 (22.8)
Gilts	75	15 (20.0)	7 (9.3)
Piglets 4 week	35	3 (8.6)	0 (0)
Piglets 6 week	40	1 (2.5)	1 (2.5)
Piglets 8 week	40	2 (5.0)	0 (0)
Piglets10 week	40	1 (2.5)	0 (0)
Piglets12 week	20	0 (0)	0 (0)
Piglets 16 week	35	1 (2.8)	1 (2.8)
Piglets 20 week	25	1 (4.0)	2 (8.0)
Fatteners 12 week	5	0 (0)	0 (0)
Fatteners over 20 week	30	0 (0)	0 (0)
Total	415	59 (14.2)	27 (6.5)

Discussion

The results of this study indicate that SIV subtypes H1N1 and H3N2 are circulating in commercial pig herds in the eastern part of Croatia. However, it is evident that the subtype H1N1 is predominant. The obtained results also demonstrated that the occurrence of antibodies depends on the age of animals and show that sows and gilts have higher antibody prevalence against SIV. This might be explained by the fact that sows represent a reservoir for continuous circulation of influenza viruses since most of them have been on the farm longer than the finishing pigs and have more exposure to infection (3). The animals sampled in this study have not been vaccinated against SIV so the seropositive animals may have been exposed to the virus at some point in their life-time from other infected pigs or even humans.

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THE IMPACT OF MATERNALLY DERIVED ANTIBODIES ON THE TRANSMISSION OF INFLUENZA VIRUS IN NEONATAL PIG POPULATIONS

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Introduction

Although serologic prevalence estimates indicate that influenza virus infections are quite common in the United States (1, 2), the epidemiology and ecology of influenza virus infections in swine populations have not been studied in detail. It is thought that once a virus is introduced into a herd it may continue to circulate and become endemic. This continued circulation is likely due to the availability of susceptible animals in a population, such as neonatal pigs (3). One measure to reduce the number of susceptible animals is through vaccination. Vaccination of breeding females is a common practice for influenza virus, with 70% of large farms in the United States vaccinating breeding females (4). Vaccination of sows and gilts should not only provide active immunity to the breeding herd, but also passive immunity through maternal antibody transfer via colostrum to the progeny. Therefore, understanding the role of maternally derived antibody on the transmission of influenza virus in neonatal pig populations is crucial. The objective of this study was to assess the role of maternally derived antibodies in reducing influenza virus transmission in swine populations.

Materials and methods

Sows from an influenza virus negative breeding herd were assigned to one of three treatment groups: homologous influenza virus vaccine, heterologous influenza virus vaccine, and no vaccine. Sows within the respective vaccine groups were vaccinated at 4-5 and 2-3 weeks pre-farrow with killed influenza virus vaccines. The homologous vaccine was created using the H1N1 challenge virus for this study and the heterologous vaccine was created using an unrelated H1N1 virus (14% nucleotide difference from the homologous vaccine).

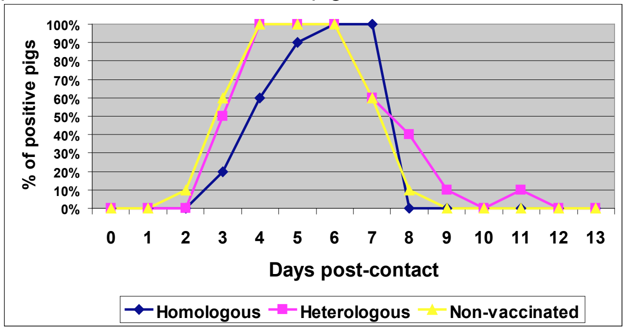
Offspring of the vaccinated sows were confirmed to be seropositive to influenza virus and purchased for use in this study. For each of three replicates, 10 pigs (3-4 weeks of age) from each of the aforementioned treatment groups were challenged with influenza virus via nose to nose direct contact with an experimentally infected pig that was introduced to each treatment group.

Nasal swabs were collected daily for two weeks following challenge and tested for influenza virus RNA via RT-PCR. The reproduction ratio (Ro) was estimated for each replicate and treatment group. Clinical signs, macroscopic and microscopic lesions, antibody titers, and nasal swab virus titers were also assessed.

Results

All contact pigs became infected within 6 days post-contact with the infected pig regardless of the treatment group (Chart 1). Timing and duration of infection post-challenge appear to differ between groups and additional replicates are in progress to further validate this finding.

Chart 1. Percentage of contact RT-PCR positive pigs by day post-contact with an infected pig



Discussion

The use of influenza virus vaccines in swine breeding herds is common. However, the impact of these vaccines on virus transmission and infection are not fully understood. This study indicates that vaccine induced maternal immunity may not be able to prevent transmission of influenza virus in population settings.

Acknowledgements

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CURRENT AND RETROSPECTIVE SEROLOGY STUDY OF INFLUENZA A VIRUSES ANTIBODIES IN BRAZILIAN PIG POPULATIONS

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Introduction

Influenza A virus (IAV) infections are endemic diseases in pork producing countries around the world. Although Brazilian swine production is expressive (4th pork producer and exporter), few reports investigated the occurrence of swine influenza virus (SIV) antibodies or isolates in Brazil. Initial studies analyzed swine sera collected from 1996-1999. Antibodies against subtype H1N1/ Texas1/77 (2.2%) and H3N2/ New Jersey/76 (16.7%) were detected by hemagglutination inhibition (HI) assay (1). The emergence of the pandemic 2009 human H1N1 influenza A virus (pH1N1) (2) raised questions about the occurrence of this virus in Brazilian swine. The objective of this work was to determine the presence of antibodies to IAV in pigs' populations before and after 2009.

Materials and methods

A serologic study on 10 pig herds using current and retrospective samples was carried out. Samples consisted of 09 commercial farms and 01 feral swine herd (176/09/2009) from Pantanal wetland in Brazil. Swine serum samples examined (359 total) were received at the Embrapa Swine and Poultry Research Center in Concordia, Brazil between 2006 and 2010. Retrospective samples were chosen from different years (before and after the occurrence of the pandemic H1N1 influenza virus), regions, and biosecurity or management levels. The selection of commercial herd's samples (not feral swine's) was based on the presence of clinical signs and influenza-like lung lesions typical of acute respiratory disease. Serologic assays included the HI (3) and the Avian Influenza MultiS-Screen Idexx ELISA (4). HI assays were used to evaluate serum samples against classic H1N1-A/sw/IA/31(AAF6/19/92) or H1N1, H3N2-A/sw/IA/8548-2 or H3N2, both purchased from NVSL-ARS-USDA and pH1N1/107b/10-3A (H1N1) or pH1N1 isolated from Embrapa's swine herds (5).

Fig. 1 – Elisa *Fig. 2 HI*

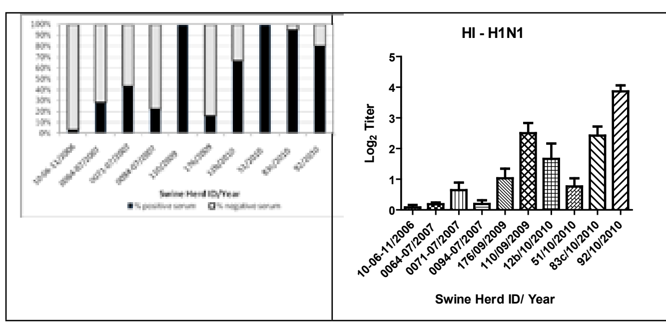
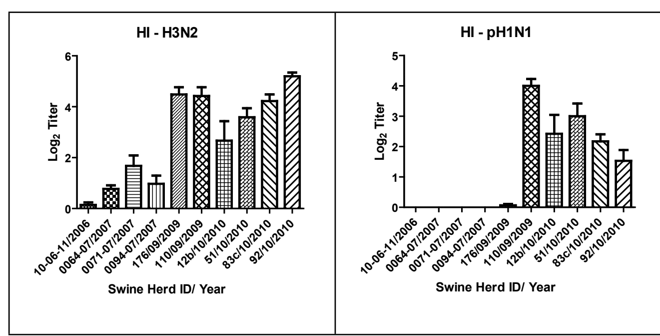


Fig. 3. *Fig. 4*



Results

In this study, a commercial ELISA developed for the detection of IAV nucleoprotein antibodies in avian species was used. The cutoff as $S/N \leq 0.673$ (positive) was used as described previously (4). Figure 1 shows Elisa results (%) and Figures 2-4 presents HI titers for IAV used in this work. Reciprocal HI titers were $\log_2$ transformed for analysis. Clearly, an increase in frequency and antibody titers from 2006 – 2010 is observed in both tests. The results show a shift in 2009, probably due to the infection of swine with pH1N1. This demonstrates a lack of specific antibodies to the pH1N1, which suggests Brazilian pigs were not fully protected against the pH1N1 from previous exposure. Besides commercial swine herds, feral swine population (176/09/2009) resulted positive to IAV antibodies by Elisa (5/31) and HI.

Discussion

This is the first detection of the pH1N1 IAV antibodies in Brazilian pigs. Although previous serologic studies have indicated the circulation of SIV in Brazilian pigs (1,6,7), no evidence of a robust immune response to IAV was observed previously. No influenza vaccines are registeredto use in Brazilian pigs herds. Based on the diversity and continuous evolution of IAV of swine, the findings of this study warn for the constant monitoring of these viruses in populations not previously diagnosed. Not only are these findings important for swine health, but they have implications for human health as well.

Acknowledgements

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SCREENING FOR PANDEMIC INFLUENZA H1N1 IN THE NORWEGIAN PIG POPULATION IN 2010

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Introduction

Up until 30th September 2009, Norway's national serological surveillance and control program has documented that the Norwegian pig population has been free from specific viral infections that are frequent in most pig producing countries. These are Aujeszky's disease (AD), transmissible gastroenteritis (TGE), porcine respiratory corona virus (PRCV), porcine respiratory and reproductive syndrome (PRRS) and swine influenza (SI) (1).

Pandemic influenza (H1N1pdm) was recorded for the first time in Norway on 10th October 2009, when an integrated pig herd sampled based on suspicion was confirmed positive for H1N1pdm (2). By 31st December 2009 routine and targeted surveillance showed that 91 out of 217 pig herds were tested positive by serology and/or real-time RT-PCR (3).

The comprehensive surveillance program covering the entire pig population was conducted also in 2010. In addition some positive herds from 2009 were monitored by repeated testing to follow the course of the H1N1pdm infection.

Materials and methods

In accordance with the program, 10 blood samples from sows in approximately 430 herds and from fatteners in 60 herds are tested annually. Herds are randomly selected from integrated and piglet-producing herds, fattening herds, all breeding (nucleus and multiplying) herds, and all sow pools. Sampling from counties is proportional to the intensity of pig farming.

Fattening pigs borne in 2010 in 38 herds that tested positive for H1N1pdm in 2009 were tested for antibodies against H1N1pdm.

Serum samples were screened for Influenza A specific antibodies using an ELISA kit (ID Screen® Influenza A Antibody Competition test, IDVET). If the results were positive or doubtful, the serum samples were re-examined using the hemagglutination-inhibition (HI) assays for antibodies against the pandemic H1N1 and European H1N1 and H3N2 serotypes according to the method described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (4).

Results

The results from the surveillance and control program in 2010 showed that 41% of the 459 tested herds were H1N1pdm positive (Table 1). Results from serological testing of sows in breeding herds indicated that 32 % of these herds were positive for H1N1pdm by February 2009 while 38 % were positive by 31.12.2010.

Testing of fattening pigs borne in 2010 in the 38 previously positive herds shows that, with the exception of four herds in one region, there was no indication of active infection with H1N1pdm in these herds. Only two new herds were tested positive for H1N1pdm by rRT-PCR in 2010, one in January and one in July.

Table 1. Results from the Norwegian surveillance and control programs in 2009 and 2010: number of H1N1pdm positive herds (% positive of herds tested) by region.

Region	2009*	2010
	No. pos (%)	
North	0 (0%)	6 (24%)
Middle	3 (3%)	48 (55%)
West	0 (0%)	5 (21%)
South-West	13 (11%)	73 (57%)
South	1 (2%)	20 (34%)
South-East	0 (0%)	20 (29%)
East	1 (1%)	18 (28%)
Total	17 (4%)	190 (41%)

*The first positive sampled 30th September 2009.

Discussion

The serological surveillance program shows that the H1N1pdm outbreak has affected about 40% of the Norwegian pig herds from October 2009 till 31st December 2010. Testing of a large proportion of sows gives a high confidence of the herd prevalence. Detailed investigation of all the received samples in 2009 revealed that the virus may have been introduced to the pig population in September 2009.

The results also indicate that there has been little transmission of H1N1pdm to new herds since February 2010. The active infection of H1N1pdm seems to have died out quickly in most infected herds. This is based on monitoring of pigs borne in 2010 in herds that were infected in 2009.

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PRESENCE OF H1N1 INFLUENZA 2009 WITHIN FOUR ESTONIAN SWINE HERDS

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Introduction

The H1N1 epidemic of 2009/2010 led only to mild symptoms in Estonia in both humans and pigs. During this period, no cases of H1N1 influenza were officially registered in the estonian swine herds.

A discrepancy between the seroincidence and clinical cases of the pandemic H1N1-2009 is known from investigations in many countries (1). Despite the lack of reports about the prevalence of H1N1 in Estonia, pigs were regularly found infected worldwide during the 2009/2010 H1N1 epidemic (2). Although the lack of clinical signs makes it difficult to estimate the true spread of a virus, it was important to find out if and how many of the estonian pigs were affected by the outbreak since even a mild infection would give good protection against further infections.

The main objective of the present work was to develop a novel ELISA assay capable of measuring the titers of anti-H1N1 in pigs. The second objective was to use the newly developed assay to determine the spread of H1N1 in the estonian pigfarms.

Materials and methods

Serum samples were collected from 96 pigs in 4 herds from different locations in Estonia.

For ELISA analysis, two commercial pandemic H1N1 vaccines – Celvapan (Baxter) and Pandemrix (GlaxoSmithKleine), both containing inactivated virions of the A/California/07/2009 H1N1 strain, were diluted without adjuvant in a sodium carbonate-bicarbonate buffer, pH 9.6, and used to coat Nunc Maxi-Sorp Immuno Plates. Samples collected at least a year before the 2009 H1N1 pandemic and PBS were used as negative controls. Non-specific binding sites were blocked with 2% casein in PBS. Both serum samples and controls were diluted by four-fold dilutions up to 1:64000 times and added to the plates followed by an overnight incubation at 4˘C. The plates were incubated with a secondary antibody (DAKO Rabbit anti-pig) for one hour at room temperature. Freshly mixed peroxidase substrate reagent (1 mM tetramethylbenzidine and 2.3 mM H_2O_2 in citrate buffer, pH 4.5) was added to the plates and incubated for 20 min at room temperature. 1M H_2SO_4 was added to each well to stop the reaction. Optical densities were measured at 450 nm.

Positive results from the ELISA were validated with a hemagglutination inhibition assay. The sera were inactivated by incubation at 56˘C for 30 min and the assay followed a standard protocol (3). HI titers were determined at the highest dilution that displayed hemagglutination activity. Specific HI activity of sera was calculated as the lowest concentration of sera that displayed hemagglutination activity.

Results

To validate our test and gain insights about H1N1 seropositivity in Estonian pig herds, blood samples from four different locations were analysed using the new assay we developed. The results were homogeneous within each farm, suggesting that the virus was well spread in a herd when present at a given site.

Animals from farm 1 had no antibodies against the pandemic H1N1 and had therefor had most probably no previous contact with the virus. This is a closed breeding farm, they do not take in animals from abroad. In contrast, the animals from farms 2, 3 and 4 were almost all seropositive with most individuals having high titers of antibodies directed against the inactive pandemic H1N1 virions present in the two vaccines. Results obtained with the antigen from two different providers were fully consistent and also confirmed by a hemagglutination inhibition assay. Remarkably, when tested for anti-H3N2 antibodies in a separate assay, most of the animals from all four herds showed significant titers against this type of influenza as well (data not shown).

Discussion

Our observations clearly indicate that pandemic H1N1 spread in 3 of the 4 tested pigfarms despite the animals not having shown any clinical symptoms, which indicates that virus present had been highly attenuated. Furthermore, we have developed a novel assay to detect the spread of H1N1 responsible for the 2009 pandemic in pigs.

Acknowledgements

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THE DISEASE ECOLOGY OF INFLUENZA VIRUS IN SWINE BREEDING FARMS

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Introduction

Influenza is a common respiratory disease in swine caused by influenza A viruses. Individual pigs infected with influenza virus shed virus for a limited period of time. In contrast, less is known about influenza virus transmission in swine populations such as swine breeding farms. An understanding of within herd transmission of influenza virus in swine populations may guide control and management practices for influenza. This may then reduce within farm transmission of influenza virus and eventually lead to the elimination of influenza virus from populations. This study was conducted to describe the disease ecology of influenza virus in infected swine breeding farms.

Materials and methods

Swine breeding farms confirmed to be endemically infected with influenza virus were selected for enrollment in this study. To determine the subpopulation responsible for maintaining influenza virus infections in breeding farms over time, cross-sectional nasal swab samplings of all subpopulations within selected breeding farms were conducted. Sixty nasal swabs were collected over time from the following four subpopulations within breeding farms: sows, gilts, neonatal pigs of 3-10 days of age, and neonatal pigs of 11+ days of age. All nasal swabs were tested for influenza virus by RT-PCR and a subset of samples were isolated and sequenced (HA gene).

Results

Influenza virus was not detected in sows or gilts at any point in time. However, influenza virus was detected in neonatal pigs on all farms sampled. Influenza virus was detected in neonatal pigs at both farms at initial sampling and at the first follow-up sampling approximately 30 days later. Results from farm A are displayed in Table 1. Samples were collected at this farm for a period of 96 days. Influenza virus was detected for a period of 27 days in neonatal pigs. Genetic sequencing (HA gene) indicated that the virus detected in pigs at day 0 had 99.5% nucleotide similarity or higher to the virus detected at day 27.

Table 1. Farm A - number and percentage of positive nasal swab samples over time

Day	Number of positive nasal swab samples by subpopulation (%)			
	Sows	Gilts	Pigs 3-10 days of age	Pigs 11+ days of age
0	0/60 (0)	0/59 (0)	1/60 (2)	6/60 (10)
27	0/60 (0)	0/60 (0)	0/60 (0)	4/58 (7)
56	NT	NT	NT	0/58 (0)
96	NT	NT	NT	0/59 (0)

Discussion

Influenza virus causes respiratory disease in pigs and has the ability to infect humans. Results of this study highlight the role of the neonatal pig in the circulation of influenza virus in swine breeding farms. Based on the results of this study, it appears that this subpopulation may be important for the continued circulation of influenza virus in breeding farms as previously described (1). It also appears that influenza virus circulation either ceases over time or becomes undetectable with the available diagnostic methods and sampling strategies.

Acknowledgements

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INFLUENZA VIRUS EPIDEMIOLOGY IN AN INFECTED WEAN TO FINISH PIG POPULATION UNDER FIELD CONDITIONS

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Introduction

Influenza is a common respiratory disease of grow-finish pigs in the United States. The seroprevalence to H1 influenza virus was estimated to be 51% in the north-central United States (1). A more recent serologic survey in the United States indicated that 71.5% of unvaccinated sites sampled were positive for either H1 or H3 influenza virus via ELISA assay (2). The aforementioned studies confírm the commonality of influenza virus in United States pig populations. Information regarding the temporal dynamics of influenza virus infections in pig populations is scarce. The objective of this study was to evaluate the epidemiology of influenza virus over time in an infected wean to finish pig population.

Materials and methods

A 5,000 head wean to finish all-in/all-out pig population (barn) was selected for this study. This population was selected as one of the two source sow farms was confirmed to be infected with influenza virus and was weaning influenza virus infected pigs.

To characterize the duration of influenza virus circulation in this population, nasal swab and oral fluid samples were collected approximately every 7-14 days until two consecutive negative sampling periods. Sixty nasal swabs were collected to detect at least one infected animal with an estimated prevalence of at least 5% and 95% confidence. One oral fluid sample was collected per every two pens (12 samples total). Day 0 was considered the first sampling, which was 2 days post-weaning and pigs were ~3 weeks of age. Nasal swab and oral fluid samples were tested for the presence of influenza virus RNA via RT-PCR.

Results

Nasal swabs collected at day 0 indicated that pigs were infected with influenza virus at the sow farm of origin. Genetic sequencing (HA gene) indicated that the virus detected in the pigs at day 0 had 99.5% nucleotide similarity or higher to the virus previously detected at the sow farm of origin. Genetic sequencing (HA gene) from days 9 and 16 also indicated that the virus detected had 99.5% nucleotide similarity or higher to the virus previously detected at the sow farm of origin. Virus isolation and genetic sequencing was unsuccessful from oral fluid samples, therefore, following day 16 genetic similarity between viruses could not be assessed.

Table 1 summarizes the results from both nasal swabs and oral fluids obtained over time. Nasal swabs collected at days 26 and 35 were negative via RT-PCR, however, a small percentage of oral fluid samples were positive via RT-PCR. This finding may be due to low prevalence of infection. Therefore, nasal swabs were not collected for the remainder of the study. Influenza virus RNA was detected in oral fluid samples until day 69 (approximately 10 weeks post-weaning).

Table 1. Number and percentage of positive nasal swab and oral fluid samples over time

Day	Number of positive nasal swab samples (%)	Number of positive oral fluid samples (%)
0	8/60 (13)	Not tested (NT)
9	28/60 (47)	12/12 (100)
16	6/60 (10)	7/12 (58)
26	0/60 (0)	3/12 (25)
35	0/60 (0)	4/12 (33)
47	Not tested (NT)	10/12 (83)
58	NT	1/12 (8)
69	NT	2/12 (17)
79	NT	0/12 (0)
96	NT	0/12 (0)

Discussion

Individual animals infected with influenza virus shed virus for only a limited period of time. This study provides evidence for extended virus circulation at the population level. Although it was unknown if the same virus persisted for the entire study period, there was evidence of influenza virus RNA detection within this specific population for a period of 69 days. This study also provides evidence that influenza virus circulation will cease in a closed population over time, however, it highlights that virus circulation in wean to finish populations is prolonged and insidious. Lastly, this study also emphasizes the fact that weaned pigs may serve as a source of influenza virus.

Acknowledgements

This study was funded by the Rapid Agricultural Response Fund, Minnesota Agricultural Experiment Station. We would also like to acknowledge the great cooperation from the Pipestone System that provided access to the grow-finish site sampled for this study.

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ESTABLISHING A SIV NEGATIVE GENETIC NUCLEUS HERD FROM TWO POSITIVE SOURCES

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Introduction

A new PIC genetic nucleus (GN) farm was stocked from two existing nucleus farms over a period of 24 weeks. Both of the stocking sources were closed systems. Each source farm was SIV positive, Farm 1 was known to be positive with a variant H1N1. Farm 2 was discovered to be positive with an H3N2 during the stocking after CFIA modified their serological test for SIV. Banked sera revealed the farm had been H3N2 positive for at least three years without clinical signs.

The objective was to stock a new GN herd from two SIV positive sources resulting in a GN herd producing SIV negative breeding stock.

Materials and methods

To confirm influenza was circulating in Farm 1, a cross-sectional serologic sampling was conducted across the growing pig population. Fifteen samples were taken from seven age groups, five to twenty-three weeks, to identify where sero-conversion was taking place. After determining infection was occurring in the nursery phase, virus isolation was successful following multiple submissions. The decision was made to develop an autogenous H1N1 vaccine[2] for use during the pending stocking of the ne GN.

Minimum wean age of piglets to be shipped for the stocking was 15 days with a maximum age of 21 days. Weekly shipments were made to the isolation unit from each source for 34 weeks on a filtered trailer. A specially designed air-filtered trailer moved each group of piglets and was dedicated to this stocking for the entire time. The piglets were isolated for 25 days after arrival. Each week, a random statistical serological testing was performed for the group to be moved to new GN's Site II (nursery and finishing). After negative results, piglets moved from isolation to the Site II nursery for another 21 days. At 14 days post entry to Site II nursery gilts were bled and serologically tested for PRRS PCR/ELISA, *M. hyopneumoniae*, and TGE/PRCV. Piglets from Farm 2 were considered sentinels for the SIV strain from Farm 1 and tested with a homologous HI test for the Farm 1 SIV.

Once all animals were delivered and cleared quarantine, animals were moved to Site 1. No new additions were made to the population for 16 weeks. Site II was emptied, washed and disinfected in preparation to receive newly weaned piglets.

Results

Farm 2 was discovered to be H3N2 positive during the stocking period. Unknowingly, H3N2 positive piglets had been delivered to the new GN site in previous shipments. In addition, serological testing indicated that the variant H1N1 Farm 1 had leaked through in spite of autogenous vaccination of the sow herd. Both viruses were present in the newly established population.

After the initial stocking, pig flow management was implemented to exploit the SIV infection timeline to allow the virus(es) time to die out and establish a negative system. Based on routine tissue submissions and available serological test, PICs new GN system is considered to be SIV negative today.

Discussion

This GN stocking was the first time PIC has stocked a new GN for reasons not related to disease elimination. The ultímate goal was to establish a new GN with minimal impact on genetic improvement. Although it was considered a lofty goal to establish a new GN SIV negative from two positive source farms with 34 weeks of introductions, the health team decided that we needed to try and establish a herd with higher health status than the source herds.

Although autogenous vaccination was effective and improved production at Farm 1, multiple sow vaccinations were not 100% effective in suppressing shedding during the entire 34 week period[3]. The Farm 1 variant H1N1 virus leaked through over time. Understanding the SIV infection timeline and managing pig flow accordingly is an effective tool in SIV elimination.

Acknowledgements

Genus Americas and PIC North America for funding this elimination attempt.

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A SEROPREVALENCE STUDY OF SWINE INFLUENZA VIRUS INFECTION IN NORTHERN ITALY PIG FARMS USING REFERENCE AND RECENT STRAINS

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Introduction

Swine influenza viruses (SIVs) represented by three different subtypes, H1N1, H3N2 and H1N2 are now co-circulating in Italy, (1). The objective of the study was to evaluate the current SIV seroprevalence in farms affected by acute respiratory clinical signs and, moreover to evaluate the importance of the strains used in the serological tests and how much this choice could influence the evaluation and interpretation of the results.

Materials and methods

In this study 25 farms (16 farrow-to-finish, 4 fattening and 5 multi-site herds) located mostly in the North of Italy were investigated. In 2009 from each farm, where no SIV vaccination was practiced, 20 sera were obtained from pigs that had been affected with acute respiratory clinical signs at least 3 weeks before. A total of 500 sera were collected and submitted to haemagglutination inhibition test (HI) (2) against reference SIV strains: H1N1 A/sw/Finistere/2899/82, H3N2 A/sw/CA/3633/84, H1N2 A/sw/Italy/1521/98 and against very recent SIV strains isolated in Italy: H1N1 A/sw/It/267505/10, H3N2 A/sw/It/312583/09 and H1N2 A/sw/It/284922/09. The results were analyzed according to the following rules: samples were considered positive for one specific subtype if the titre was ≥1/20; one farm was considered positive against one given subtype if at least 2 sera showed an HI titre ≥1/20 against this subtype. Risks of misinterpretation due to cross-reactivity between SIV strains were decreased by considering non-positive, for one subtype, a serum with a positive titre, if the difference with the titre to the other subtype was higher than 3 log2. Data were analyzed with Fisher's Exact Test.

Results

Globally, 76% (19 out of 25) of farms tested were positive for at least one reference SIV subtype, while the percentage rises to 92% (23 out of 25) when recent field SIV strains were used. Considering the 23 SIV positive farms, it was observed that some farms were positive for more than one SIV subtype and, in particular, 13 were positive for H1N1 and H1N2 subtypes, 16 to H1N1 and H3N2 subtypes and 10 to H1N2 and H3N2 subtypes. The serological results are summarized in table 1 and 2. The differences between the data collected were statistically significant.

Table 1. Serological results on sera from 25 farms tested against reference and recent SIV strains using HI test.

Tested strain	N° positive farms	% positive farms
H1N1 2899/82	11	44%
H1N1 267505/10	23	92%
H3N2 3633/84	8	32%
H3N2 312583/09	16	64%
H1N2 1521/98	5	20%
H1N2 284922/09	13	52%

Table 2. Comparison of the HI titres obtained using the reference and recent SIV strains.

Tested strain	<20	20	40	80	160	≥320
H1N1 2899/82	454	20	14	8	2	2
H1N1 267505/10	312	52	53	43	22	18
H3N2 3633/84	446	12	21	14	5	2
H3N2 312583/09	328	30	41	34	30	37
H1N2 1521/98	483	13	3	1	0	0
H1N2 284922/09	373	42	46	28	9	2

Discussion

This serological survey confirms that SIV infection is frequent in Italian pig farms having shown respiratory disorders. SIV infection is enzootic in swine producing regions of Italy and the highest prevalence was found when the sera were tested against recent isolates, representative of the SIV strains circulating in the area. This observation confirms that the use in HI tests of updated strains adapted to the local epidemiological situation is a major asset for improving the accuracy of SIV surveillance programmes as observed in a previous serological study performed in France (3).

Acknowledgements

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OUTBREAK OF H1N2 SWINE INFLUENZA IN ITALY: A FIELD CASE

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Introduction

According to recent virological and serological surveillance studies, swine influenza prevalence in Italy is high (1). The objective of this paper is to describe a field case of flu in an Italian farm and to assess the estimated cost of this outbreak.

Materials and methods and results

This study was run in a 350-sow farrow-to-finish farm affected by PRRS, Aujeszky disease (AD) and *Mycoplasma hyopneumoniae* (Mh).

- Symptoms and diagnosis

February 2010: 3 sows aborted, no diagnosis could be established after serological and virological analysis.

October 2010: an "influenza-like" outbreak occurred in 6 to10-month old fattening pigs and in growers: fever, feed consumption decreased by 20%. Nasal swabs analysed by PCR were negative for SIV. Few days later, reproduction disorders occured at different stages of gestation (abortions, early farrowings) and on newborn piglets. Serological tests performed in October on sows evidenced high level of anti SIV antibodies by HI tests.

In November, "influenza-like" symptoms on fattening pigs were observed: fever (41-42°C), decrease of feed intake and growth rate. Flu diagnosis was established with the Flu Detect® test (Synbiotics, Lyon, France) on 8-month fattening pigs: all 5 nasal swabs tested were positive. Some days after, 6 Virocult® (Medical Wire & Equipment, Corsham, Wiltshire, England) nasals swabs were performed on 5 growers and 1 aborted sow and 3 lungs from growers were collected. Lungs were positive for Mh and swine influenza but negative for all other investigated respiratory pathogens. From the 5 grower swabs, a strain belonging to the H1N2 subtype was isolated.

- Vaccination programme and treatments

Early 2010, sows were mass injected with 2ml of a combined vaccine against AD and flu (H1N1 and H3N2 subtypes). A booster was injected 3 months later, in April 2010.

Mid October, sows were injected with GRIPOVAC®3 (immunization against 3 different subtypes of Flu: H1N1, H3N2 and H1N2) and a vaccine against AD.

PRRS infection was also suspected by clinical observations, but no confirmation could be shown after virological analysis (serum and organs) on sows and piglets.

Sows were treated with Paracetamol for 7 days when clinical signs appeared. Grower and finishers affected by clinical signs were treated with acetyl salicylic acid and antibiotic.

- Economic impact of the flu outbreak

Table 1: Economic impact of the outbreak:

	Details*	€*
Treatments costs	Collective treatments: Sows: € 1000 Growers: € 2700 Finishers: € 5200 Individual injections: € 1130	10030
Feed costs "saved" due to mortality	Fatteners: 300kg x €23	-6900
	Growers: 40kg x €40	- 1600
Abortions	550 piglets x € 30**	16500
Mortality (n total=142)	7000 kg x € 1.440	10000
Quartering costs	7 tons of carcasses x € 144	1000
Growth rate decrease	2500 pigs x 0.5kg/day x 7days x € 1.25	15625
Total		44655
Total/ sow	350 present sows	127

*Usual losses recorded in this farm during same period were removed. ** Market price of a weaned piglet.

Discussion

This Italian farm experienced a flu outbreak in all age groups for almost 2 months. The diagnosis allowed to exclude the role of PRRSV, Aujeszky virus and PCV2 infections, and to confirm swine influenza. The cost of the SIV outbreak in this farm reached €127 per sow which was considerable and higher than other published estimations (2,3).

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IMPACT OF FEEDING SPRAY-DRIED PLASMA TO PIGS CHALLENGED WITH SWINE INFLUENZA VIRUS

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Introduction

Spray-dried plasma (SDP) is widely used as a feed ingredient in swine diets to provide beneficial effects on production parameters such as gain, feed efficiency, and reduced diarrhea scores. Research indicates that the beneficial effects of plasma are related to modulating the inflammatory response both locally in the intestine and systemically (1). Studies have demonstrated improvements in survival and viremia during respiratory challenges when animals consumed SDP (2, 3). The objective of the present study was to determine the impact of feeding SDP as a supplement in the diet or spray-dried serum (SDS) in the water on the outcome of experimental swine influenza virus (SIV) infection in pigs.

Materials and methods

Eighty-four 21 d old piglets obtained from a herd free of SIV were randomly assigned to three treatments. Treatments were: 1) control – no SDP; 2) SDP – fed 8% SDP in the feed from d 0 to 28; 3) SDP/SDS – fed 8% SDP in the feed from d 0 to 28 and 2% SDS in the water from d 15-28. All pigs were challenged with SIV 40776 1992 (H1N1) $10^{7.5}$ EID_{50}/mL on d 21. Clinical symptoms were determined daily. Blood samples were collected on d 0, 21, and 28. During the challenge period (d 21-28), nasal swabs were collected. On d 28, pigs were euthanized and necropsied. Pathological examination, bronchoalveolar lavage (BAL) fluid, and lung lesion scores were determined at time of necropsy.

Results

During the challenge period, no differences (P > 0.05) were noted on clinical scores including rectal temperature, cough, and body condition. Respiration rates tended (P < 0.10) to be decreased on d 25 and 27 in the SDP/SDS group compared to control. Lung lesion scores were not different (P > 0.05) and averaged 11.5, 10.9, and 13.2%, respectively for control, SDP and SDP/SDS groups. No differences (P > 0.05) were noted in serology, nasal swabs, and BAL fluid. From d 14-21, average daily gain was increased (P < 0.05) when pigs consumed SDP/SDS compared to control, while pigs consuming only SDP were intermediate. After challenge, average daily gain was reduced in all treatments; however, no differences (P > 0.05) between treatments were noted during the challenge period. Due to the challenge resulting in lower lung lesion scores and less clinical symptoms than anticipated, a subset of 56 pigs with higher lesions scores (greater than 5%) were evaluated. In the subset, lung lesions were reduced (P < 0.05) in pigs consuming SDP in the feed compared to controls (20.0 vs. 13.7%), while pigs consuming SDP/SDS was intermediate (17.1%). During the challenge period (d 21-28), average daily gain was increased (P < 0.05) in pigs consuming SDP/SDS compared to controls (96 vs. 212 g/d), while pigs consuming SDP were intermediate (156 g/d).

Discussion

In the current study, pigs challenged with SIV resulted in lower lung lesions than anticipated. Similarly, clinical symptoms were minimal. In this model, consumption of SDP or SDP/SDS was not different than controls. However, when a subset of pigs exhibiting higher lung lesions were evaluated lung lesions were less for the group fed SDP. Challenge with SIV reduced average daily gain of all group, but to a lesser degree for the SDP/SDS group. In summary, pigs consuming SDP or SDP/SDS had numerically better performance during a subclinical SIV challenge. When pigs exhibited greater lung lesions and consumed SDP or SDP/SDS performance was improved to a greater magnitude.

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OCURRENCE OF SWINE INFLUENZA IN FARMS FROM MEXICO

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Introduction

Swine influenza virus (SIV) belongs to the *Orthomyxoviridae* family; it is easily spread among pigs and it has world-wide prevalence. The virus has a high genetic and antigenic variability due to the elevated rate of mutation. The main subtypes circulating in pigs are H1N1, H3N2 and H1N2. Outbreaks on farms are characterized by high morbidity with less than 5% of mortality. In addition, SIV represents a zoonotic risk for people in close contact with infected pigs (2). For this reason, epidemiological surveillance of influenza viruses on swine farms is a valuable tool for public and veterinary health.

The aim of this study was to provide a preliminary overview on epidemiological data for SIV in pigs from Mexican farms.

Materials and methods

A cross-sectional study was conducted in 15 commercial farms selected from major producers in Sonora, Mexico, during October 2008 to March 2009. The sample size (n=150) was estimated considering a 30% prevalence of swine influenza infections in farms and a confidence level (CL) of 95%.

Nasal swabs and blood samples were collected from unvaccinated pigs. Viral RNA was extracted from each nasal swab sample and rRT-PCR assay targeting the M gene was performed. Positive samples were further subtyped with conventional RT-PCR using previously described primers (3). Further cDNA sequencing and phylogenetic analyses were conducted. A commercially available ELISA kit (IDEXX) was used for the detection of SIV-specific antibodies.

To estimate statistically significant differences between SIV prevalences, we conducted proportions for hypothesis test, and squared Chi (X^2) to infer differences for virus detection with a CL of 95%.

Results

Our results showed that 25 out of 150 samples were positive to type M gene (16.6%). Six of them could be subtyped by RT-PCR: four samples were positive for H1 and two were of the H3 subtype. Sequence analysis was done in four samples (three H1 and one H3); the results showed the viruses circulating in Sonora are phylogenetically closer to North American (NA) strains. At the hemagglutinin level, the H1 showed an 89% nucleotide identity with Sw/NE/123/77(H1N1) and Sw/ONT/57561/03 (H1N1). The analysis of the H3 gene demonstrates the highest identity (97%) with SW/MN/SG-00234/05 (H3N2).

Serological testing showed that 47% of the samples were positive for H1N1, 62% for H3N2 and 38% for both. Seropositivity was observed in all farms with at least one positive sample. The seroprevalence for H1N1 showed an age-dependent decreasing trend ($p<0.05$), while the H3N2 subtype demonstrate a decrease from 1 to 12 weeks, and a slight rise through the 13th week.

Discussion

The phylogenetic analyses of the viruses showed that H1 and H3 from viruses identified in Sonora farms belong to North American swine lineage. It was noted that the identity is higher among the H3 subtypes than among H1 virus, this may be because the mutation rate is higher in H1 than H3 (4). Serological studies have also documented that the occurrence of SIV is higher for H1 than H3 (5). The opposite has been observed in Mexico (6) and our results are in agreement with these findings.

The decay of humoral response to H1N1 in weaning pigs could be related to the decreasing of passive immunity conferred by sows to the piglets through the colostrum feeding (2). As for H3N2 subtype, no differences are found among age, this could mean that we are detecting a more active circulation of this subtype in farms; it is important because most of the epizootic outbreaks in high swine producer region of NA are caused by H3N2 (7).

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OUTBREAK OF SWINE INFLUENZA, SUBTYPE H1N2: A CASE REPORT AND ITS FINANCIAL CONSEQUENCES

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Introduction

The most common swine influenza viruses (SIVs) currently circulating in the European countries are H1N1, H3N2 and H1N2 (1). The economic importance, the within-herd infection dynamics and the clinical impact of the disease on piglets are, however, not fully elucidated. The objective of this study was to evaluate the economical impact of a Swine Influenza outbreak in a farrow-to-finish herd in UK.

Materials and Methods

This case took place in a 2-site production system, with a breeding unit (BU) with 760 sows and a different site for the feeding herd unit (FU). These units are part of a large integrated system. Every 6 weeks, 100 kg maiden gilts are introduced in batches of 40-50 animals and are served after 9 weeks. Piglets are weaned at approximately 8 kg and are moved off-site to the FU. The costs resulting from controlling secondary infections and minimizing the impact of SI were assessed based on the cost of production for this integrated system, the pork price and the production system economic data at the time of the outbreak.

Case description - diagnosis

At the end of 2008, production parameters and health status deteriorated in several batches in the BU. In the meantime, the FU suffered an outbreak of *Haemophilus parasuis*, piglet vaccination against Glasser's disease was initiated. Over 6 months, gilts showed various undefined symptoms including respiratory and reproduction signs.

In February 2009, 12 non paired serums, taken from maiden gilts, on site for 5 weeks, were analysed and the presence of H1N2 and H1N1 was confirmed (9/12). Analysis of two ill thriven piglets and material of one abortion did not confirm viral involvement.

At the end of March, a major outbreak occurred. Within several days all areas of the unit were affected with a morbidity of approximately 70%, presenting cough, dyspnoea, nasal discharge, lethargy and decreased feed intake (especially at farrowing). Five sows died in the first days of the outbreak, 16 days after the start of the outbreak, H1N2 was confirmed on 10 out of 12 serums. A nursing piglet was examined post mortem for diagnosis of *Haemophilus parasuis,* lung tissue histology suggested SIV involvement.

Another respiratory disease outbreak emerged 14 days after the initial outbreak. The clinical picture milder in the adults was severe in suckling piglets, especially from 10 days prior to weaning, with coughing, lethargy, vomiting and increased pre-weaning mortality. On the FU, 23 serums tested by PCR, excluded PRRS virus involvement. Three weaned pigs were examined post mortem; *Mycoplasma hyopneumoniae* was detected by PCR. H1N2 virus was isolated from two of these pigs. These data confirmed that H1N2 was the initiating pathogen.

Results

The cost of this acute H1N2 SI outbreak was assessed and calculated for the breeding and feeding herd, table 1. The costs of this H1N2 outbreak were calculated at a total of £ 18 per sow, reflecting only the losses to the integrator, this excludes the losses by the breeder and finisher units as contractors.

Table 1: Analysis of the cost of an outbreak of H1N2

	Herd Details	£
BU	Death of 5 pregnant sows (litter and sow carcass loss and disposal and sow feed)	2,213
BU	7.7 % drop on the conception rate	417
BU	Lower litter size (0.5 piglets) during 21 days	211
BU	Pre weaning mortality increase of 3%	236
BU	Water soluble and in feed medication	2,600
FU	63% mortality increase over 2 months (90 extra dead pigs at approximately 14 kg live weight and their disposal)	4,058
FU	In feed and water soluble medication	3,905
	Total cost	**13,640**

Discussion

Gilts presented undefined symptoms for several months. SIV was detected through serology. Weeks after, swine influenza, H1N2 was confirmed.

The financial impact of a virulent SIV outbreak was estimated in this system at approximately £18 per sow. SI vaccines, not available at the time of the outbreak would have been a useful tool controlling infection and preventing economic losses.

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PREVALENCE OF H1N1, H1N2 AND H3N2 SWINE INFLUENZA VIRUS IN POLISH HERDS IN 2010 COMPARING TO THE PERIOD 1998-2009

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Introduction

Swine influenza (SI) remains one of the most important and worldwide prevalent viral respiratory diseases in pig population, causing economic losses in swine industry. It is also considered an important factor in public health concern. Therefore the regular monitoring of the epidemiological situation concerning SI is of great necessity. The surveillance program for SI was initiated in Poland in 1998. The aim of this paper is to present epidemiological situation concerning SI in Poland in 2010 and its retrospective analysis over 13 years period.

Materials and methods

Serological survey. In total 38311 sera were tested, including 36139 pig sera taken in slaughter houses located in all 16 districts of Poland; 1477 blood samples taken from heart or pleural cavity of wild boars, hunted in 11 districts; and 695 human sera taken from volunteers (554 from producers or animal caretakers and 141 from veterinarians). In 2010 5250 pig sera were tested. The samples were tested according to the standard procedure in HI test against the reference viruses representing 3 subtypes (H1N1 - A/sw/Belgium /1/98, H3N2 - A/sw/Flanders/1/98, H1N2 - A/sw/Gent/ 7625/99). All necessary reference sera, virus and RBC controls were included in each test. The sample was considered positive when the antibody titer was ≥1:20.

Virological survey. In total 731 samples taken from pigs demonstrating influenza-like symptoms were tested, including: 589 nasal swabs, 82 lung tissue samples and 60 blood samples. The biological material was sampled in 71 farms located in 12 districts. Additionally 4 foetuses from aborting sows demonstrating influenza symptoms were analysed. Virological investigations included: virus isolation in 10 days old embryonated SPF chicken eggs and MDCK cells, detection of viral RNA by Real Time PCR based on M1 gene, molecular subtyping in mPCRs based on HA and NA genes, and phylogenetic analysis. Five passages of each specimens were done.

Results

In serological investigation of the pig sera it was clearly demonstrated that H1N1 subtype is dominated in Poland. In 2010 the seroconversion to this subtype reached 30.1% of tested sera. The percentage of animals possessing antibodies against the mentioned subtype during the period of 1998-2010 varied, with the range from 5 to 30%. Within last years we observed significant increase of seroconversion against H1N1. We also evidenced that the H1N1 subtype circulate at the higher frequency in the West part of Poland, in pig densely area where the pig production is better developed. The survey for H1N2 subtype performed in 2010 demonstrated 11.9% of seropositive samples. It also varied over the time, ranging from 2 to 12%. The seroconversion to H3N2 strain in 2010 was detected in 15.7% of tested sera. The significant increase (about 5-fold) of antibodies against H1N2 and H3N2 subtype was evidenced within last 5 years. In contrast to H1N1, H3N2 subtype circulate at the higher frequency in the East part of the country, where the small private farms are mainly located and pig production is less developed. Additionally mixt infection with 2 or even 3 subtypes were noticed. The situation among wild boars was similar to that observed among pigs but the prevalence of antibodies was significantly lower. Interestingly the highest occurrence of antibodies against the tested SIV strains was evidenced in human. Seroprevalence for H1N1, H1N2 and H3N2 antigens reached 45.4%, 34% and 62.4%, respectively.

Using conventional virology method 54 SIV isolates were obtained. RNA of SIV was detected in 149 (20%) samples originated from 49.3% farms. In molecular subtyping 94% of isolates were determined as H1N1 subtype, 3.7% as H3N2 subtype and 1.9% as H1N2.

HA gene of Polish isolates have been derived from contemporary strains of “avian-like” SIV. M1 gene may have originated from avian influenza viruses. H3N2 strain was located in swine cluster, in the main prevalent European group of H3N2 isolates (A/PortChalmers/1/73-like lineage).

Discussion

Due to the fact that infections of pigs with H1N1 virus were described in most European countries intensively producing pig we can state that epidemiological situation of Polish pig herds concerning SI is similar to that observed in European countries. The prevalence of virus specific antibodies is smaller and is entirely different from that observed in the USA (66.3%-100%) and Asia (45%). In last 5 year the significant increase of SIV specific antibodies was evidenced.

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PANDEMIC SWINE INFLUENZA VIRUS (H1N1) 2009 FROM PIGS IN CUBA

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Introduction

Influenza is a zoonotic viral disease that represents a health and economic threat to both humans and animals worldwide (1). In April 2009, a new swine-origin H1N1 influenza virus was identified circulating in humans and rapidly reached pandemic magnitude (2). Pigs are documented to allow productive replication of human, avian, and swine influenza viruses (3). Therefore, monitoring of influenza virus in pig population is critical to know the influenza viruses circulating in a country, reducing the potential serious economic damage as well as exposure of humans to the virus. The objectives of the current study were to determine the presence of the novel pandemic influenza (H1N1)/2009 virus in pigs as well as other swine influenza viruses in Cuba as part of a surveillance FAO Project (TCP/RLA/3206).

Materials and methods

A total of 157 nasal swabs and 81 lung samples from sixteen swine farms all across the country were collected in November 2010. The nasal swabs and the lung samples were taken from pigs that showed respiratory disorders and interstitial pneumonia, respectively. We processed five nasal swabs and lung samples for virus isolation in embryonated chicken eggs and MDCK cells selected from the real time RT-PCR positive samples. Haemagglutination test was performed as described at the OIE Manual Viral RNA was extracted from nasal swabs and 10% tissue homogenates by using QIAamp Viral RNA Mini Kit (Qiagen) according to the manufacturer's directions. Total cDNA was produced by reverse transcription (RT) using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, USA). A Real-Time RT-PCR for the detection of swine influenza virus and identification of the novel N1 subtype to differentiate pandemic A/H1N1 was performed.

Results

Swine influenza and pandemic H1N1 Real-Time RT-PCR results are summarised in Table 1. Swine influenza viral genome was detected in 66 out of 238 (27.7%) samples collected from pigs with respiratory disorders and/or interstitial pneumonia. A total of 24 samples out of 238 (10%) yielded positive results for pandemic influenza A/H1N1. Swine influenza infections were detected in 15 out of 16 (93.8%) swine farms and pandemic H1N1 influenza infections were detected in 5 out of 16 (31.3%) farms evaluated. Influenza virus was successfully isolated from positive Real-Time RT-PCR samples. The isolates were positive by haemagglutination and Real-Time RT-PCR assays.

Table 1. Influenza virus genome detected in a total of 238 nasal swabs and lung samples.

Virus	No. Positive	% positive
Swine Influenza	66	27.7
Pandemic H1N1	24	10

Discussion

The emergence of pandemic H1N1/2009 influenza directly demands verification of whether such viruses are circulating among the pig population. The results of the current study showed the potential presence of the novel pandemic influenza H1N1/2009 in Cuban pig populations and the occurrence of influenza infections in pigs with respiratory disorders and interstitial pneumonia. The novel H1N1/2009 virus is not known to be circulating widely among swine. However, there are some reports confirming the detection of the pandemic influenza H1N1 virus in pig herds all over the world (4). This study provides essential epidemiological information for risk analysis and decision making in the control and prevention of the disease. Sequencing and molecular analyses of the isolates are currently in progress.

Acknowledgements

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FIRST ISOLATION OF SWINE INFLUENZA VIRUS, SUBTYPE H1N2 IN SWINE IN THE NETHERLANDS

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Introduction

Infections with Swine Influenza Viruses (SIV) are common in commercial pig farms in Western Europe. Traditional subtypes H1N1, H3N2 and H1N2 are responsible for these infections in most of the European countries. Data of serologic screenings are confirming the circulation of all three subtypes of SIV in The Netherlands (1). By using serologic data, a history of SIV circulation can easily be confirmed in farms, but detection of the virus in clinically affected animals is the best way to demonstrate the causal link between SIV infection and the observed clinical symptoms. Detection of the virus can be performed by a quick test (Flu DETECT® Swine, Synbiotics, Lyon), which shows presence of Influenza virus type A. The quick test is not subtype specific. Thereafter, the subtype needs to be confirmed by PCR or virus isolation. To our knowledge, isolation of the SIV subtype H1N2 has not yet been reported in The Netherlands. This report describes the consequences of a confirmed SIV H1N2 outbreak in The Netherlands in a farrow-to-finish farm.

Materials and methods

The present report describes an outbreak of SIV H1N2 on a well-managed 330-sows farm in one of the most densely populated pig regions in The Netherlands. Sows are vaccinated with GRIPOVAC®, a bivalent SIV vaccine containing SIV subtypes H1N1 and H3N2; every gestation, a booster vaccination is performed at 13 to 14 weeks of gestation.

At the end of January 2011, an increased mortality rate, sudden death and coughing were observed in the post weaning piglets. In the finisher stage, clinical symptoms included moderate fever (39,8-40,6°C), anorexia, coughing, wheezing, sneezing, weakness and sudden death of three finisher pigs. Four days after the beginning of the symptoms, 50 percent of the finisher pigs were affected. The problems in the post weaned piglets were resembling to *Streptococcus suis* symptoms, and therefore some pigs were sent in for necropsy. The clinical symptoms in the finisher stage were highly suggestive for SIV infection. Nasal swabs were taken from 6 animals and tested by the Flu DETECT® Swine test, provided by the MERIAL Flu detection KIT. Additionally, six VIROCULT® (Medical Wire & Equipment, Corsham, Wiltshire, England) swabs of the same animals were sent in for virus isolation to the Laboratory of Virology (Ghent University, Belgium) where isolation was performed on Madin Darby Canine Kidney (MDCK) cells and also on embryonated eggs.

Results

One out of the six samples tested clearly positive in the quick test, while for 2 other samples, the results were more difficult to interpret. The 3 other samples showed negative results in the quick test. Virus isolation showed positive results for all 6 swabs tested. Identification of the isolate revealed the SIV H1N2 subtype to be responsible for this outbreak of acute respiratory symptoms in the farm.

Figure 1: The results of the quick test

Table 1: Economic consequences of the outbreak

Medication costs	350 €	350
Death of three finisher pigs	3 x 150 €	450
Growth retardation	1000 finisher pigs x 1,5 kg feed x 7 days growth stop x 0,25 €/kg food	2625
Total		3425 €

Discussion

This SIV H1N2 outbreak confirms the need for a complete protection against all 3 subtypes present in The Netherlands when vaccination is performed. The calculation of the financial consequences (Table 1) is most probably a severe under estimation, due to the fact that long term respiratory problems which are common after SIV outbreaks are not yet taken into account. The diagnosis in this farm confirms the usefulness of the MERIAL Flu detection KIT, to optimize the diagnosis of SIV in the field. Making a correct diagnosis can also help to reduce the use of antibiotics; since this case confirmed again that no antibiotic treatment was needed.

Acknowledgements

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TRANSMISSION OF PANDEMIC INFLUENZA A/H1N1 2009 VIRUS TO PIGS IN NEW CALEDONIA, AN INSULAR REGION PREVIOUSLY FREE FROM SWINE INFLUENZA VIRUSES

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Introduction

The first human infections with the multi-reassortant H1N1 influenza virus (pH1N1) responsible for the 2009 pandemic were not related to a contemporary infection in the swine population. However, genomic sequence analysis revealed that pH1N1 contains a combination of genes of swine origin that had never been identified previously (1). Data from experimental infections of pigs and experience of natural outbreaks in swine farms worldwide have shown that porcine populations are fully susceptible to this virus and able to sustain uninterrupted transmission chains (2, 3). Considering pig susceptibility to pH1N1, monitoring activities were conducted in New Caledonia, an overseas French island region. New Caledonia was previously shown to be free from swine influenza viruses (SIV) and has experienced a major epidemic wave in humans during the austral winter 2009.

Materials and methods

Seroprevalence surveys were carried out on breeders in December 2009. 300 sows were sampled from 21 herds. Based on the results from this initial seroprevalence study, an additional prospective study was carried out at the slaughterhouse from June to August 2010. 166 pigs from 22 herds born in 2010, thus after the 2009 epidemic phase in humans, were randomly sampled. Blood sample, nasal swab and lung sample were taken from each tested animal.

Sera were first analyzed using the ID Screen_ Influenza A Antibody Competition ELISA kit (ID-Vet). They were further analyzed with an hemagglutination inhibition (HI) test for antibodies against four virus lineages: pH1N1 (A/California/4/09) and European H1N1, H3N2 and H1N2 SIVs (A/Sw/Finistere/2899/82, A/Sw/Flandre/1/98 and A/Sw/Scotland/410440/94, respectively). When an animal tested positive (HI titre >20) against several viruses, it was considered positive against the virus with the highest HI titre, as cross-reactivity may exist.

Nasal swab supernatants and lung samples were analyzed using Influenza A M-gene real-time RT-PCR assays (4). Positive samples were further analyzed towards pH1N1 using specific RT-PCR assays targeting the H1 gene or the N1 gene from this virus (4). Virus isolation was performed on MDCK cell cultures.

Results

No clinical influenza-like syndrome was reported in pig herds in New Caledonia at the time of the epidemic wave in humans, or just after. However, analyses by ELISA of sera taken on breeders late 2009 revealed the presence of antibodies against Influenza A virus in 80% of the tested farms (77% of positive animals). HI tests showed that these antibodies were related to pH1N1 in 81% of farms (86% of tested animals). The mean HI titre was 166. Within-herd prevalence was high, varying between 60 to 100%.

Further investigations carried out from June to August 2010 led to the detection of 11% of seropositive animals towards pH1N1 among this generation of fattening pigs. Thus, 27% of the tested herds were hypothesized to have been infected with pH1N1 during the first half of the year 2010. RT-PCR analyses revealed the presence of Influenza A genome in 2 of the 22 investigated herds. However, no virus isolate was obtained, probably due to limited amounts of viral particles in the positive biological samples. Finally, sows that were already sampled in December 2009 were shown to be still seropositive towards pH1N1 in August 2010. They come from 3 different herds and their mean HI titres were, at that time, as high as those obtained 8 months before.

Discussion

The overall results support the hypothesis of a sustain, a year after the epidemic in humans, of the almost asymptomatic circulation of pH1N1 in pigs in New Caledonia. They illustrate the human-to-pig pH1N1 transmission and its adaptation to swine. In addition to direct animal health concerns, pH1N1 circulation in pigs may have consequences for human health, by increasing the reservoir of this virus.

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PRIMER-PROBE ENERGY TRANSFER PCR FOR DETECTION OF ALL SIV SUBTYPES AND PANDEMIC H1N1 2009 VIRUS

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Introduction

The first objective of this study was to develop a novel singleplex real-time PCR assay to quantify the viral loads of RNA of avian, human and porcine influenza viruses. The second objective was to combine the Real-Time included probe assays with Real-Time based on the temperature melting curve assay followed by PCR detection system originally developed by Rasmussen et al (3). This method was evaluated to detect mutable potential of swine influenza viruses (SIV).

Materials and methods

PCR primers were designed using matrix (M) gene nucleotide alignment enclosed Polish and European SIV isolates. The comparisment were made to determine sites with the lowest number of genetic mismatches. Forward primer was labeled with Oregon Green as a donor dyes and Cy5 as an emittor.

The M genes of H1N1 field strain (A/Swine/KPR9/2004) strains was amplified by RT-PCR and the amplification products were cloned into the pCR 2.1 vector using a dual-promoter TOPO TA cloning kit (Invitrogen). The number of RNA copies was calculated. Tenfold dilutions of the RNA transcripts, ranging from 1 to 10^{10} copies/µl, were prepared. Other subtypes like H1N1, H1N2 and H3N2 representing by A/Sw/Bel/1/98, A/Sw/Eng/96 and A/Sw/Fl/1/98, AIV and pH1N12009 IV strains and by the field isolates were also included. The reaction efficiency was determined by the calculation of the correlation coefficient (R^2).

Results

Primer-Probe Energy Transfer hybridization probes were designed for SIV, and the PCR product 121 bp long were detected by melting curve analysis and confirmed by electrophoresis separation. Specific melting curves with temperature around 74ºC, were observed only with swine, avian and human influenza viruses.

For all Polish isolates melting profile was reached in 74.8°C. Sequence differences between the PCR product and hybridization probes resulted in shifts in the melting temperatures (74.8°C for reference H1N1 virus and 71.7°C for one of H1N2 reference viruses) were detected. Moreover the shift was observed in melting temperature using RNA extracted from two different subtypes of H1N2 viruses: A/Sw/Scot/410440/94 and A/Sw/England/17394/96. Single or few mutation within the probe sequences can shift Tm curve. According to internal gene – matrix, used in PCR amplification, no correlation between different subtypes of SIV and Tm specificity was found.

As it was shown the correlation between C_T values and copy numbers was linear over the range 10^1 to 10^5 copies and with a efficiency of 109%, therefore the assay is optimal. Using Cy5 probe dye and FAM- or OregonGreen-pimer, in terms of M gene copy number, the limit of detection for the H1N1 subtype was 10^1 gene copies/µl of *in vitro* transcribed RNA.

Discussion

Numerous PCR-based methodologies for quantifying influenza virus RNA copies have been described in various formats. Results from many studies confirm that influenza A virus is constantly evolving and these mutations may have important implications in clinical diagnosis and possibly treatment, management, and vaccine development. Pri-ProET SIV PCR assays showed a considerable species-specificity and sensitivity of detection with 10 genomic copies for matrix gene. In case of our study, we observed a loss of viral subtype discriminatory ability using Tm analysis, within just the same subtype – H1N2 but two different isolates. PCR-based systems do allow detect a polymorphisms but also may lose sensitivity over time due to high mutability of the virus, and this may hinder laboratory diagnosis. Fortunately, the longer length of the FRET probes, compared to that of alternative probe formats (e.g., TaqMan), and the ability to perform Tm analysis allows for continued detection of influenza A isolates with the Pri-ProET assay, although subtyping cannot be performed (2). Similar goal of the PriProEt assay was formed by Balka et al with the assay for detection of PRRSV (1).

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DEVELOPMENT AND DURATION OF POSTVACCINAL HUMORAL IMMUNITY AGAINST SWINE INFLUENZA AFTER VACCINATION OF PIGS IN THE PRESENCE OF PASSIVE IMMUNITY

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Introduction

Influenza virus (IV) infections are a common and important cause of respiratory disease in pigs. In some countries, vaccination of pigs is a popular method of control of swine influenza (SI) (1, 2). Most SI vaccines is used for sow vaccination what may results in long lasting maternal immunity (MI) in piglets (1). Regarding this, vaccination of weaners and fatteners may be difficult to combine with vaccination of sows because of prolonged MI.

In this study the influence of MI on the initial development and duration of postvaccinal humoral response (HR) after vaccination of pigs against SIV was evaluated.

Materials and methods

Fourteen seronegative sows and their litters were used. Seven sows were vaccinated, at 6 and 2 weeks before parturition, with the use of commercial inactivated bivalent vaccine (containing H1N1 and H3N2 IV). Blood from piglets was taken at 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18 and 20 weeks of life (wk) and tested for Ab against H1N1 and H3N2, with the use of HI test.

Group	Age of vaccination		
	MDA-positive pigs		**MDA-negative pigs**
M(+)C	non-vaccinated	**M(-)C**	non-vaccinated
M(+)4+8	4 and 8	**M(-)4+8**	4 and 8
M(+)1+4	1 and 4	**M(-)1+4**	1 and 4
M(+)1+8	1 and 8	**M(-)1+8**	1 and 8
M(+)8+10	8 and 10	**M(-)8+10**	8 and 10
M(+)8+12	8 and 12	**M(-)8+12**	8 and 12

Results

HR was observed in all sows after vaccination. None of the non-immune pigs born from non-immune sows had Ab against H1N1 and H3N2, indicating, that no infection had occurred during the period of study. Maternally derived Ab (MDA) in the sera of non-vaccinated piglets born to immune sows (M(+)C) were above level considered positive until about 13 wk for H1N1 and until about 9 wk for H3N2. The kinetics of decline of HI titers was different for Ab against H1N1 and H3N2. The mean titre of MDA against H1N1 was over the 60 till 12 wk, while mean titre against H3N2 decreased under 60 as early as at 6 wk. The active HR against H1N1 in pigs vaccinated in the face of MDA were developed only in pigs from groups M(+)8+10 and M(+)8+12. In pigs vaccinated at earlier ages, levels of Ab did not increase after vaccination. For H3N2, seroconversion was noted in all but one of the groups (M(+)1+4). However, in this group after final vaccination the level of Ab stopped to decrease and remaining relatively constant (HI about 40) till 10 wk. All MDA(-) pigs developed H1N1 and H3N2–specific HR that were different in kinetics and magnitude, to responses developed by the MDA(+) pigs. The H1N1-specific HR were the highest
in pigs that were vaccinated for the first time at 4 or 8 wk.Two wk after final vaccination the mean HI titres were significantly different from this observed in group M(-)1+4 ($p<0.01$). In pigs from groups M(-)1+4 and M(-)1+8 the slight seroconversion were also noted. The H3N2-specific HR was found in all vaccinated piglets. The significant difference between mean HI titres 2 weeks after final vaccination was observed only between groups M(-)1+4 and M(-)8+10 ($p<0.05$).

Discussion

Results of the present study demonstrated that piglets from group $M_{(+)}$8+12, starting from 14 weeks of pigs life, had the highest mean HI titre against H1N1. In all vaccinated groups the antibody levels until the end of the study were higher than in the non-vaccinated control, what indicates that in all vaccinated groups an active HR must have taken place, even if this did not result in an observable rise of the titres. It seems that passive immunity interfered with development of postvaccinal humoral immunity. The influence of age on the initial development and duration of postvaccinal HR was also observed in present study. In pigs vaccinated for the first time at an age of 1 week the mean HI titre against H1N1 2 wk after final vaccination were lower ($p\leq0.05$) than in pigs vaccinated when they were older. The same was also true for H3N2.

According to our results, piglets born to seropositive and seronegative sows should not be vaccinated with the first dose before 8 wk. Moreover, age of vaccination was associated with differences in magnitude of Ab responses to SIV. Caution should be exercised in extrapolating results from this study to other vaccines against SI, other vaccination protocols and others herd-specific data on the levels of MDA

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COMPARISON OF EFFICACY OF SUBUNIT-BASED PANDEMIC H1N1 INFLUENZA VIRUS VACCINES BY SUBLINGUAL AND INTRAMUSCULAR ADMINISTRATION IN SWINE MODEL

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Introduction

Due to CNS-associated adverse effect of intranasal delivery system, the sublingual (S.L) route of influenza virus vaccines has been studied for effective route for mucosal and systemic immune responses. Therefore, pandemic influenza virus-based subunit vaccines were prepared for pigs and their efficacy was compared by intramuscular or sublingual administration which was first trial in swine species.

Materials and methods

Ten SPF-Sinclair pigs were arranged into 4 groups: 3 vaccinated and challenged groups (B, C and D) and one non-vaccinated and challenged group (A). The vaccinated groups were divided by vaccine type and inoculation route: Group B, subunit vaccine (HA1+mCTA/LTB) and sublingual (S.L); Group C, subunit vaccine (HA1+mCTA/LTB) and intramuscular (I.M); Group D, inactivated vaccine and I.M. The vaccination was performed two times for 2 weeks interval and all pigs were challenged with pandemic influenza virus (A/swine/GCVP-KS01/2009 (H1N1)) and monitored for clinical signs, serology, viral shedding and histopathology.

Results

After vaccination, HI titer was higher (320) in group D than the other vaccinated groups (40-80). The motility and feed intake were reduced in group C. Both viral shedding and histopathological lesions were reduced only in group B and D.

Figura 1. Comparison of HI titers for pandemic influenza virus during the study.

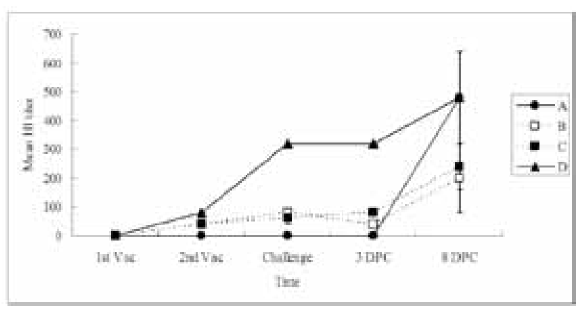

Figura 2. Comparison of mean lung scores, 2 weeks after challenge of pandemic influenza virus isolate (A/swine/GCVP-KS01/2009 (H1N1)).
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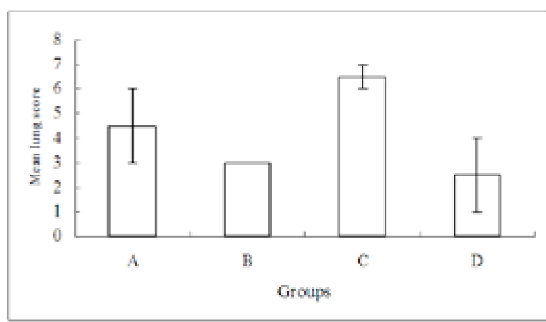

Discussion

The S.L administration of pandemic influenza virus vaccines was first tried for pigs in this study. The subunit vaccine (HA1+mCTA/LTB) was effective showing similar protection with conventional inactivated whole viral vaccine (I.M) when administered using S.L route rather than intramuscular administration. This study provided possibility of S.L vaccination in human application through swine experiment.

Acknowledgements

This work was supported by National Agenda Project grant from Korea Research Council of Fundamental Science & Technology and KRIBB Initiative program (NTM3111013).

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P.195

SENSITIVITY OF ORAL FLUIDS TO DETECT INFLUENZA VIRUS IN VACCINATED AND NON-VACCINATED POPULATIONS

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Introduction

The use of oral fluids for disease monitoring and surveillance of porcine viral pathogens is increasing in North America. Porcine oral fluids are now routinely used for detecting porcine reproductive and respiratory syndrome virus from field samples. Detection of influenza virus in oral fluids collected from experimentally infected pigs has also been reported (1,2).In this abstract, we report the use of oral fluids to detect influenza A virus in populations of pigs to determine the sensitivity of detection at the population level and the probability of virus detection in vaccinated and non-vaccinated pigs.

Materials and methods

Eighty eight 3-week-old influenza free pigs were placed in separate rooms and assigned to one of three treatments in groups of 10: a) non-vaccinated (NV), b) vaccinated with a commercial, heterologous vaccine (HE), and c) vaccinated with an experimental, homologous vaccine (HO). There were 2 replicates of NV and 3 replicates for the HE and HO groups. Two wks after the second vaccination, an influenza-infected pig ("seeder pig") was challenged with A/Sw/IA/0239/2004 H1N1 and placed in contact with the treated pigs (1 pig/replicate) 48h post infection. Nasal swabs were collected daily from each pig and a rope placed daily in each pen to collect oral fluids. Oral fluids were wrung from the ropes and refrigerated at 4°C for 24 h to allow separation of debris from the fluids. Viral RNA from all samples was assayed for the presence of influenza by RRT-PCR. A pen was considered positive if at least one nasal swab was positive.

Statistical analysis were performed to compare PCR results from oral fluids and nasal swabs using Fisher's Exact Test, as well as a logistic regression model to test the predicted probabilities for influenza detection in oral fluids (SAS System, SAS Inst., Cary, North Carolina, v 9.2).

Results

A pen was considered influenza positive if at least one nasal swab was positive. A total of 1155 individual nasal swabs were collected and 13.2% were positive which corresponded to 46 pens considered positive. One hundred five oral fluid samples were collected throughout the study of which 37 were positive. Overall 43.8% of pens were detected positive but only 35% based on oral fluids. The overall analytical sensitivity of oral fluids was 80%. In the NV group, 80% of the pen collections had at least one positive pig and the same pen collections were positive by oral fluids. In the HO group, oral fluids were only positive when the seeder pigs were positive and 20.5% of the pen collections were positive but only 14% were positive by oral fluids. In the HE group, 54% pen collections were positive and 41% were positive by oral fluids. Overall PCR results from nasal swabs and oral fluids were strongly associated (Fisher's exact test, $p<0.001$). The predicted probability of detecting influenza in oral fluids from a pen with 11 pigs was 50% if 1 pig was infected, increasing to 99% if 2 pigs were infected and to 99.9% if 3 to 10 pigs were infected.

Discussion

Results from this study indicate that pen based collection of oral fluids is a sensitive method to detect flu in pigs. Although in this study sensitivity of oral fluids was slightly lower than sensitivity of nasal swabs, collection of individual swabs in all pigs is not always possible and it certainly would be an impossible task in the field. In addition, oral fluids are also a sensitive method to detect influenza when within group prevalence is low and when the pigs have been vaccinated.

Acknowledgements

The study was supported in whole or in part with the federal funds from the NIH, National Institute of Allergy and Infectious Diseases, Department of Health and Human Services under the contract No. HHSN266200700007C, and the Swine Disease Eradication Center at the University of Minnesota.

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P.196
DETECTION OF INFLUENZA VIRUS IN AEROSOLS FROM INFECTED PIGS

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Introduction

Influenza A virus is a primary respiratory pathogen of pigs (1). Infected pigs shed the virus through nasal secretions for a period of approximately five to seven days and transmit the pathogen through nose-to-nose contact (2). Influenza virus can also be transmitted through aerosols although the relative importance of this route of transmission remains contentious (3). In addition, literature in regards to detection of airborne influenza virus is scarce. The objective of this study is to validate procedures to detect airborne influenza from pigs and determine the patterns of aerosol excretion in experimentally infected pigs.

Materials and methods

Two replicates of 11, 7-week-old pigs were housed in two separate rooms at the University of Minnesota Animal Isolation facility. All pigs originated from an influenza negative pig farm in Minnesota. One pig ("seeder") from each group of 11 pigs was infected with H1N1 influenza A virus in a separate room and commingled with the other 10 pigs when shedding in this pig was confirmed 48 h post challenge. Pigs were in contact for 8 days after commingling.

Both nasal swabs and air samples were collected daily. Nasal swabs were collected from all pigs and samples tested by RT-PCR. A cyclonic collector, capable of collecting 400L of air per minute, was used for air collection. The collector was located in the room, 74 cm from the wall and 89 cm from the floor (just above the pigs). Ten milliliters of minimum essential media (MEM) supplemented with 2% bovine serum albumin was added to the cyclonic collector vessel and the collector was allowed to run for 30 minutes. Three air samples were collected approximately every 8 hours each day for 8 days or until the study was terminated. A sterile syringe was used to extract the MEM after the 30 minute sampling period and the sample was then stored in a sterile plastic tube for testing. Air sample fluids were tested for influenza A virus RNA through RRT-PCR. An attempt to quantify the amount of viral particles in the air was done though virus titration of PCR positive samples on MDCK cell culture.

Results

Both seeder pigs and all in-contact pen-mates were confirmed to be shedding influenza virus for at least 4 days and a maximum of 6 days during the 8 day study period. A total of 41 air samples were collected. Out of those, 25 (52%) were positive to influenza virus. In both groups, influenza virus was detected for the first time 2 days after commingling when 4 out of 11 pigs were shedding virus. All air samples were positive for influenza from day three after commingling until the first sample on day seven. During these days, all pigs in the room tested positive by individual PCR (e.g. influenza A virus RNA positive by nasal swab RRT-PCR).

Titration of PCR positive air samples yielded negative results.

Discussion

The relative importance of aerosol transmission in transmitting influenza virus remains contentious. Our results indicate that influenza virus excreted from infected pigs can play a role in influenza transmission in pigs. The results indicated that influenza virus was aerosolized and detected in air samples collected with a cyclonic collector device. Detection of virus in air samples correlated with shedding patterns in infected animals and presence of active contact transmission. In addition, there was a strong correlation between detection of the virus in the air and number of positive pigs in the group. In this study we could not recover viable virus from the air samples and those results are under further evaluation.

Our results validate procedures that indicate that infected aerosols can be found in environments housing influenza infected pigs. More studies are needed to further understand the relevance of influenza airborne transmission under field conditions.

Acknowledgments

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P.197

RAPID ON-SITE DETECTION OF SWINE INFLUENZA VIRUSES AND THE PANDEMIC H1N1. COMPARISON OF TWO RAPID TESTS AND RT-PCR

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Introduction

The swine origin of the new pandemic H1N1 (pH1N1) influenza A virus, and the role of swine in the epidemiology of animal and human influenza, make swine influenza (SI) diagnosis and surveillance of paramount importance. The aim of this study was to conduct a comparative evaluation of the performance of two rapid tests and Real Time Polymerase Chain Reaction (RT-PCR) for SIV and pH1N1 with viral isolates and respiratory specimens.

Materials and methods

A new rapid influenza antigen detection test kit (kit A) (Flu DETECT, Synbiotics, Lyon, France), and an existing kit (kit B) (Directigen A+B, Becton Dickinson, NJ, USA) were compared in their limit of detection with egg-derived H1N1, H3N2 and H1N2 SIVs, and the pH1N1 strain in titrated dilutions (hemagglutinin [HA] titer). A positive test result was visually detected as colored lines. The line in positive tests was arbitrarily scored with "+" symbols based on the colour intensity (from + to +++). Invalid results (absence of the control band) were also recorded. This set of samples was also tested by an RT-PCR assay adapted from Fouchier (1). A positive result was recorded when cycle threshold (Ct) values were ≤ 40 provided it showed a melting curve consistent with the expected melting temperature.

To evaluate the diagnostic performance of both test kits, a total of 56 respiratory specimens (from a frozen archive) from confirmed or suspected cases of SI infection were analyzed. Samples comprises pig lung homogenates (n=51) and nasal swabs (up to 3 pooled samples) (n=5). All samples were also tested by RT-PCR as described above. The performance parameters for each of the two kits were calculated versus RT-PCR (considered as reference method) in 2 X 2 contingency tables.

Results

The analytical and clinical performance of the two kits evaluated is summarized in Tables 1 and 2, respectively. Both rapid test kits and RT-PCR detected all four antigens tested, although differences in sensitivity were observed. Fourteen of the 56 respiratory specimens (25%) failed to be absorbed into the device within several minutes with kit A (invalid results). No invalid results were recorded with kit B.

Table 1. Comparison of two rapid tests and RT-PCR for the detection of SIVs and pH1N1 in dilution series of influenza A viruses.

Dilution Rate	Kit	Virus Subtype*				PCR/ Ct value†
		H_1N_1	H_3N_2	H_1N_2	pH_1N_1	
10	**A**	+++	+++	+++	+++	+ / 17,1
	B	+++	+++	+++	+++	
100	**A**	++	++	++	++	+ / 19,1
	B	++	++	++	++	
1000	**A**	v w+	w+	w+	w+	+ / 24,9
	B	+	+	+	+	
2000	**A**	-	v w+	w+	-	+ / 24,7
	B	-	+	+	w+	
4000	**A**	-	-	v w+	-	+ / 25,3
	B	-	w+	w+	v w+	
6000	**A**	NT	-	-	-	+ / 26,9
	B	NT	v w+	v w+	v w+	
8000	**A**	NT	-	-	NT	+ / 27,4
	B	NT	-	v w+	NT	
10000	**A**	-	-	-	-	+/ 28,9
	B	-	-	-	-	
100000	**A**	-	-	-	-	+ / 33,0
	B	-	-	-	-	

* = A/sw/spain/53207/2004 (H1N1) (HA titer 256), A/sw/spain/40564/2004 (H1N2) (HA titer 256), A/sw/spain/87902/2008 (H3N2) (HA titer 512), and A/spain/JR/2009 (pH1N1) (HA titer 256); † mean threshold cycle (Ct) values; w+ = weak positive; v w+ = very week positive; - = negative; NT = Non-tested.

Table 2. Performance of two rapid tests for the diagnosis of SI with respiratory specimens.

Kit	Kit Performance (95% confidence interval)			
	Sensitivity	Specificity	PPV†	NPV*
A	53,7 (38,7-67,9)	93,3 (70,2-98,8)	95,7 (79,0-99,2)	42,4 (27,2-59,2
B	61,0 (45,7-74,3)	93,3 (70,2-98,8)	96,2 (81,1-99,3)	46,7 (30,2-63,9)

† PPV: positive predictive value, * NPV: negative predictive value.

Discussion

Results presented here indicate that RT-PCR has better analytical and clinical performances than rapid antigen detection kits. Moreover, Flu DETECT works similarly than Directigen A+B, although it shows lower sensitivity and a significant proportion of test failures. Rapid influenza test kits are easy to perform and allow on-site identification of SIVs. However, when used as the sole test, false negative results are a major concern. To facilitate early intervention and allow disease control and surveillance, the use of rapid tests in combination with RT-PCR and virus isolation would be necessary.

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P.198

INFLUENZA A ANTIBODY DETECTION OVER TIME USING A NUCLEOPROTEIN BLOCKING ELISA

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Introduction

Classically, hemagglutination inhibition (HI) assays have been used to detect anti-influenza A virus (IAV) serum antibodies. However, HI antibody titers are greatly affected by the challenge virus in the assay. Commercial indirect ELISA assays are available, but provide poor diagnostic sensitivity for detection of antibodies against contemporary IAVs (1, 2). The objective of this study was to evaluate a blocking ELISA based on the detection of serum antibodies against the IAV nucleoprotein (NP) using serum samples of known. Diagnostic performance (Se/Sp) and factors associated with the assay response (vaccination, challenge isolate, and DPI) were analyzed in an experimental challenge model.

Materials and methods

A total of 82 PRRSV-, IAV-, and *M. hyopneumoniae*-negative, 3-week-old weaned piglets were isolated for 30 days and confirmed negative to these pathogens by repeated testing. A subset (n=28 pigs) was vaccinated (**VACSTAT**) against IAV using a commercial multivalent vaccine (Flusure XP®, Pfizer Animal Health).

After isolation, pigs were transported to a research facility and randomly assigned to one of 3 treatment groups: (1) inoculation with IAV Ohio ’07 H1N1 (n=35; of these n=11 vaccinates) (**INOC**), (2) IAV Illinois ’09 H3N2 (n=35; of these n=11 vaccinates), or (3) negative control (n=12; of these, n=6 vaccinates). Serum samples were collected weekly DPI -7 to +42, randomized, and assayed using AI MultiS-Screen Ab ELISA (IDEXX® Laboratories). Receiver operator characteristic analysis (MedCalc® v9.5.2.0) was used to calculate the optimized cut-off and associated diagnostic sensitivity (Se) and specificity (Sp) estimates. Proc GLIMMIX (SAS® 9.2, SAS Institute Inc., Cary NC) was used to analyze a repeated measurement mixed model.

Results

Based on results from 279 negative and 288 positive samples, the optimized cutoff was S/N ≤ 0.60 (AUC = 99.3). This cutoff resulted in an overall estimated Se of 95.5% (95% CI: 92.4%, 97.6%) and Sp of 99.6% (95% CI: 98.0%, 99.9%. Table 1 shows Se/Sp of the SN assay by DPI for unvaccinated animals. Table 2 shows the percent of ELISA-positive vaccinated pigs by DPI. A MANOVA determined that all three factors, DPI, VACSTAT, INOC as well as their two- and three-way interactions had a statistically significant (p<0.002) effect on the ELISA S/N value. Differences in the duration of ELISA positivity were observed in unvaccinated+challenged vs. vaccinated+challenged groups and were significant at DPI +42.

Table 1. Se/Sp (%) of NP ELISA (S/N ≤ 0.60) by DPI and IAV subtype in unvaccinated pigs

	Day post inoculation (DPI)							
	-7	0	+7	+14	+21	+28	+35	+42
H1N1	0	0	95.8	100	100	91.7	91.7	100
H3N2	0	0	87.5	91.7	95.8	95.8	100	95.8
Total	0	0	91.7	95.8	97.9	93.8	95.8	97.9
Specificity (not vaccinated, not IAV inoculated)								
Neg	100	100	100	100	83.3	100	100	100

Table 2. Percent of vaccinated pigs positive by NP ELISA (S/N ≤ 0.60) by DPI and IAV subtype

	Day post inoculation (DPI)							
	-7	0	+7	+14	+21	+28	+35	+42
H1N1	↓	↓	100	100	90.9	90.9	90.9	81.8
H3N2			90.9	100	90.9	100	90.9	81.8
Total	13.6	27.3	95.5	100	90.9	95.5	90.9	81.8
(vaccinated but not IAV inoculated)								
Neg	16.7	50	0	0	0	16.7	0	0

Discussion

A cut-off of S/N ≤ 0.60 provided excellent diagnostic specificity and sensitivity for detection of antibodies against IAV. This work is in general agreement with results reported by Ciacci-Zanella et al. (3). DPI, INOC, and VACSTAT are important to NP ELISA responses. The NP ELISA has excellent potential for detection of IAV infection across a range of subtypes.

Acknowledgements

This work was supported in part by the National Pork Board (#09-193), Pfizer® Animal Health and IDEXX® Laboratories, Inc. A special thanks to the Serology department of the Iowa State University Veterinary Diagnostic Laboratory. Contemporary virus isolates were generously provided by Drs. Amy Vincent (National Animal Disease Center) and Marie Gramer (University of Minnesota).

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P.199
DETECTION OF INFLUENZA A VIRUS IN ORAL FLUID RING TEST SAMPLES BY VIRUS ISOLATION

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Introduction

The H1N1 pandemic of 2009 highlighted the swine industry's limited surveillance of influenza A virus (IAV). Addressing this short-coming is complicated by the relative diagnostic insensitivity of IAV detection based on nasal swabs. More recently, oral fluid (OF) has been shown to be an excellent surveillance tool for several swine respiratory viruses (1,2,3). Preliminary data suggested that IAV detection in the field might be improved through the use of OF samples. The objective of this study was to determine whether diagnostic laboratories could recover IAV from OF by virus isolation.

Materials and methods

In this study, OF was inoculated ("spiked") with either contemporary H1N1 or H3N2 influenza virus for submission to diagnostic laboratories in a "ring test" format. To prepare samples, 5.4 liters of OF were collected from 4 PRRSV-, IAV-, *M. hyopneumoniae*-, and ADV-negative sows, centrifuged, and pooled. To create ring test samples, 8 10-fold serial dilutions were prepared from Ohio '07 H1N1 and Illinois '09 H3N2 stock solutions. The initial concentrations of the two isolates were 1 x 10^1 $TCID_{50}$ different, but this was not determined until the inocula were back-titrated. Samples were randomized, grouped into sets of 180 samples (10 samples from each of the 8 dilutions of each virus plus 20 negative OF samples), frozen at -80C and submitted to 6 U.S. diagnostic laboratories using overnight delivery. Laboratories were contacted to confirm samples arrived frozen. Each laboratory performed virus isolation according to their established protocol.

Results

Results of attempts at virus isolation are reported for H1N1 (Figure 1) and H3N2 (Figure 2) by dilution. One negative control sample among 20 provided was reported positive in one laboratory. Analyses and comparisons of methods are in progress.

Discussion

IAV can be isolated from spiked OF samples, but success rates differ among laboratories, particularly as virus concentration declines. Preliminary analysis suggests that successful isolation of virus is dependent upon laboratory protocol and technique. Field samples must be assessed to confirm the applicability of these observations.

Figure 1. Isolation of H1N1 virus from oral fluid samples by 10-fold dilution (percent positive)

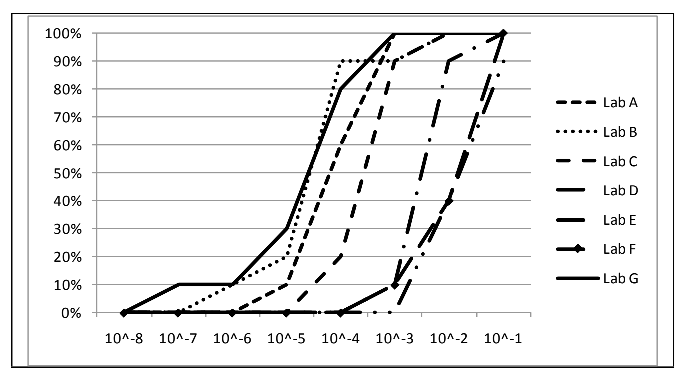
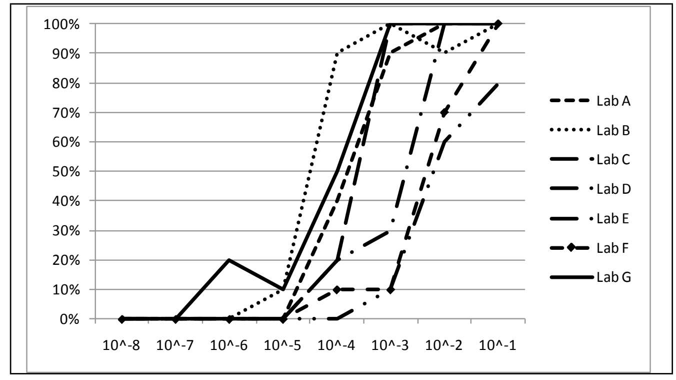


Figure 2. Isolation of H3N2 virus from oral fluid samples by 10-fold dilution (percent positive)



Acknowledgements

This work was supported by USDA/APHIS (NCAA: 10-9100-1314-CA).

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P.200

DETECTION OF INFLUENZA A VIRUS IN ORAL FLUID RING TEST SAMPLES BY RT-PCR

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Introduction

The H1N1 pandemic of 2009 highlighted the swine industry's limited surveillance of influenza A virus (IAV). Addressing this short-coming is complicated by the relative diagnostic insensitivity of IAV detection based on nasal swabs. More recently, oral fluid (OF) has been shown to be an excellent surveillance tool for several swine respiratory viruses (1,2,3). Preliminary data suggested that IAV detection in the field might be improved through the use of OF samples. The objective of this study was to determine whether diagnostic laboratories could repeatedly detect IAV from an OF ring test by rt-PCR.

Materials and methods

In this study, OF was inoculated ("spiked") with either contemporary H1N1 or H3N2 influenza virus for submission to diagnostic laboratories in a "ring test" format. To prepare samples, 5.4 liters of OF were collected from 4 PRRSV-, IAV-, *M. hyopneumoniae*-, and ADV-negative sows, centrifuged, and pooled. To create ring test samples, 8 10-fold serial dilutions were prepared from Ohio '07 H1N1 and Illinois '09 H3N2 stock solutions. The initial concentrations of the two isolates were 1 x 10^1 $TCID_{50}$ different, but this was not determined until the inocula were back-titrated. Samples were randomized, grouped into sets of 180 samples (10 samples from each of the 8 dilutions of each virus plus 20 negative OF samples), frozen at -80C and submitted to 8 U.S. laboratories using overnight delivery. Laboratories were contacted to confirm samples arrived frozen. Each laboratory performed rt-PCR using the method(s) of their choice.

Results

Rt-PCR results are reported for H1N1 (Figure 1) and H3N2 (Figure 2) by dilution. Seven of the 8 laboratories reported as positive at least one of the 20 negative control samples (Figure 1). Analyses and comparisons of methods are in progress.

Discussion

IAV can be detected by rt-PCR from OF samples, as previously reported (1), but success rates differ among laboratories, particularly as virus concentration declines. Preliminary analysis suggests that successful rt-PCR detection is dependent upon laboratory protocol. Field samples must be assessed to confirm the applicability of these observations.

Figure 3. PCR detection of H1N1 virus from oral fluid samples by 10-fold dilution: 8 labs, 12 different methods (percent positive)

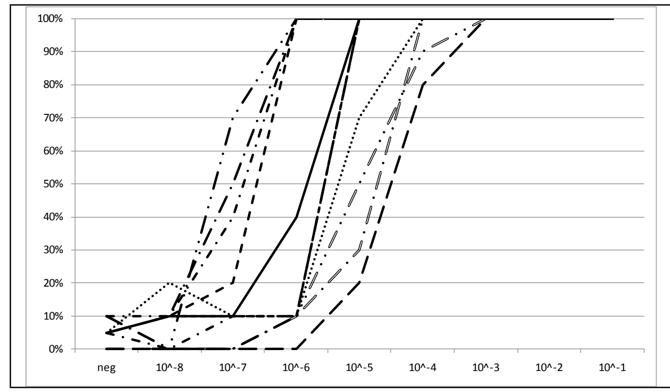
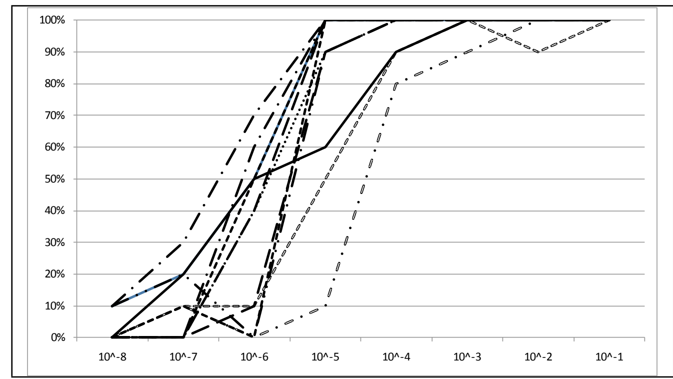


Figure 2. PCR detection of H3N2 virus from oral fluid samples by 10-fold dilution: 8 labs, 12 different methods (percent positive)



Acknowledgments

This work was supported by USDA/APHIS (NCAA: 10-9100-1314-CA).

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Posters

Emerging and Re-emerging Viral Infections

(P.201 - P.257)

P.201

IDENTIFICATION OF A 9-MER CTL PEPTIDE WITH PROTECTIVE POTENTIAL AGAINST AFRICAN SWINE FEVER VIRUS

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Introduction

African Swine Fever (ASF), a disease notifiable to the OIE, is provoked by African Swine Fever Virus (ASFV). There is no vaccine or treatment against ASF and the only measures to control the disease rely on the stamping out of all pigs within the area of the diagnosed ASF-case. Little is known about the mechanisms involved in protection against ASFV although there are evidences indicating that both humoral and cellular responses can play a role in protection. Fruit of the work made in our laboratory developing experimental DNA vaccines against ASF, a new CTL peptide has been identified in the viral hemagglutinin (HA). Preliminary immunization experiments using this peptide demonstrate the protective potential of this 9-mer peptide.

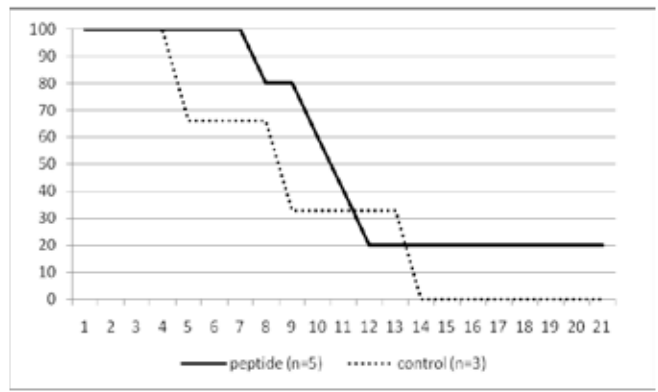
Materials and methods

Computational CTL predictions allowed designing a peptide library covering the p54, p30 and HA genes of ASFV. These peptides where used individually to stimulate PBMCs from pigs immunized with a DNA vaccine encoding these three antigens and capable to resist the lethal challenge with the E75 strain (10^4 UHA). The specific stimulation was measured by an IFN-γ ELISPOT assay. Skin fibroblasts derived from same surviving pigs were used to confirm the specificity of the CTL epitopes. To confirm the protective potential of these peptides, they were used to immunize pigs (50µg of peptide in the presence of Freund´s Adjuvant) and a fortnight after the second immunization; all animals were challenged with a lethal dose of E75. Surviving rates were recorded.

Results

From the 46 HA-peptides synthesized, only three, all mapping within the HA (1) polypeptide, scored above background. A clear immunodominance phenomenon was observed since most of the detectable response was driven against the F3 peptide (>400 positive-cells per million of PCMCs). The specificity of the response was confirmed by demonstrating the capability of the F3 peptide to bind to SLAI-bearing skin fibroblasts. Furthermore, peptide coated fibroblasts, efficiently stimulated the induction of IFN-γ when co-incubated with the autologous PBMCs. After immunization with two doses of the F3 peptide, 20% of the pigs (1 out of five), survived the lethal challenge with E75 (Figure 1). Surviving pig cleared the virus from day 10 showing a total recovery thereafter.

Figure 1. Percentage of surviving animals from both control and peptide-immunized groups.



Discussion

Our results clearly demonstrate the protective potential of CTL responses against ASFV, even in the absence of antibodies. We honestly believe that the protection rate obtained with a single 9-mer CTL peptide (still we do not know the SLA I restricted haplotype it recognizes), could be dramatically improved by adding new ASFV determinants, thus covering the broad SLA I haplotype heterogeneity present in the pig population. We are currently searching for new CTL epitopes within the whole ASFV genome, a complex tax taking into account it is 170kb in length.

Acknowledgements

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DISRUPTION OF NUCLEAR ORGANIZATION DURING THE INITIAL PHASE OF AFRICAN SWINE FEVER VIRUS INFECTION

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Introduction

The nucleus of the infected cell plays an essential role during most viral infections, even for viruses traditionally considered cytoplasmic.

Despite African swine fever virus (ASFV) has been considered as a purely cytoplasmic virus, some authors have shown evidences of an early stage of nuclear replication (1,2). Until now, little is known about the mechanisms of viral DNA egression from the nucleus to the cytoplasm of the infected cells or the nuclear modifications produced during this early ASFV nuclear stage.

The objective of the present work was to study the possible disruption of nuclear envelope and to characterize the modifications suffered by the host nucleus during the initials steps of ASFV infection.

Materials and methods

Three dimensional immune-FISH and immunofluorescence experiments imaged by confocal microscopy were performed on uninfected and infected Vero cells with Ba71V ASFV strain at different times post-infection (4, 6, 8, 12, 18hpi). The probe used for DNA FISH has been previously described (3). Nuclear proteins were detected using specific antibodies against lamin A/C, nucleoporin p62, RNA pol II (H5: recognize the hyperphosphorylated form at serine 2), splicing speckles (SC-35) and nucleophosmin/B23.

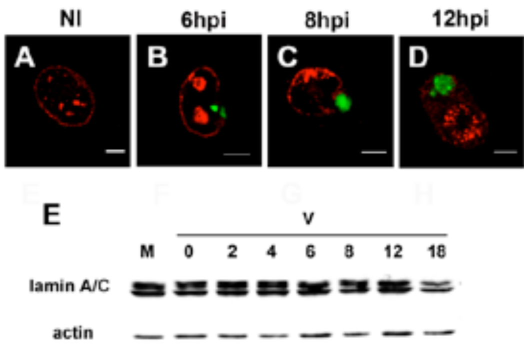
Furthermore, Western blot analyses were performed using cells extracts from either uninfected or infected Vero cells at different times post-infection. Equal quantity of protein was probed with specific antibodies against lamin A/C and β-actin.

Results

At early time post-infection (6hpi), the nuclear distribution of lamin A/C was clearly disrupted, at the sites where the viral DNA was located (Fig.1B). At later time points, this disruption was still evident (Fig.1C) and lamin A/C was also found diffusely distributed within the cytoplasm of the infected cells (Fig.1D).

During the ASFV infection, enlarged foci of lamina aggregates were also localized throughout the non-nucleolar regions of the nucleoplasm (Fig.1B-C). This nuclear lamina redistribution was not accompanied by an increment of expression (Fig.1E). Furthermore, a similar redistribution was observed with the SC-35 and RNA pol II nuclear markers in the infected cells. At late times post-infection, enlarged splicing speckles were observed in the nucleus of the infected cells. At the same time, the RNA pol II was recruited to very well defined areas of the nucleoplasm to finally disappear from 12hpi.

Figure 1. Lamin A/C disruption during ASFV infection. 3D-immunoFISH of uninfected (panel A) and ASFV-infected Vero cells (panel B-D). Lamin A/C is shown in red. ASFV DNA is shown in green. (E) Western blot analysis of cells extracts from mock-infected (M) and ASFV-infected Vero cells (V) using an antibody against nuclear lamin A/C.



Discussion

Our results demonstrate, for the first time, the disruption of the nuclear envelope during ASFV infection suggesting a mechanism of viral DNA egression from the nucleus to the cytoplasm of infected cells. A similar mechanism has been well characterized during herpesvirus infection (4).

Furthermore, the reorganization of nucleoplasmic lamin A/C has been also described during herpesvirus infections and in the presence of transcription inhibitors (4,5). The reorganization of lamin A/C, the SC-35 and the RNA pol II in enlarged nucleoplasmic foci suggest a possible mechanism to block the cellular transcription early during ASFV infection. These results indicate a more serious implication of the nucleus during ASFV infection.

Acknowledgements

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A BAYESIAN MIXED MULTIVARIATE LOGISTIC REGRESSION MODEL FOR THE EVALUATION OF THE AFRICAN SWINE FEVER RISK FACTORS IN SARDINIA.

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Introduction

African swine fever (ASF) is one of the most economically-devastating viral diseases of pigs. After its first description in 1921, ASF rapidly spread from Sub-Saharan African countries into Europe, Central and South America, and recently (2007), into Georgia, Armenia, Azerbaijan, and the Russian Federation. ASF was eradicated from The Americas and Western Europe, with the exception of Sardinia, where the disease has been endemic since 1978. Although a rigorous EU-supported ASF eradication program has been in place in Sardinia since 1993, ASF outbreaks are still reported on an annual basis in the island. It has been hypothesized that the ASF eradication program in Sardinia has failed because of certain factors that promote the disease such as extensive premises with nil or insufficient biosecurity measures, contact between wild boars and domestic pigs, illegal production/ trade of pigs, and use of waste to feed pigs. However, there is no evidence that soft ticks play any role in the maintenance or spread of ASFV-infection in Sardinia (1, 2). Noteworthy, studies aimed at assessing the association between ASFV infection with epidemiological factors in Sardinia have not been recently published in the peer reviewed literature. There is a need to better understand the nature and extent at which epidemiological factors affect the risk for ASF in Sardinia. In this study, Bayesian modeling was used to explore the association between ASF occurrence in Sardinia from 1993 through 2009 and hypothesized risk factors for the disease. Results will help to improve the effectiveness of ASF-eradication program in Sardinia and in other ASF-infected territories.

Materials and methods

A Bayesian mixed multivariable logistic regression model was used to quantify the strength, as indicated by the values of the regression coefficients (β), of the association between ASF outbreaks reported per Comune and epidemiological factors hypothesized to influence disease status in Sardinia. The response variable was whether or not the Comune reported ASF outbreaks from 1993 through 2009 (yes, no). Candidate variables to fit the model were each of the 46 epidemiological factors for which information was collected and their second-order interactions. Spatially unstructured and structured random effects were included in the model to account for unmeasured factors that were randomly distributed and that had some spatial structure, respectively. The model was fitted using WinBUGS with 100,000 iterations (first 500 samples were burned out). The best fitting model was assumed to be the one with the lowest deviance information criterion (DIC) value.

Results

The model that best fitted the data (DIC=231.04) included the number of open fattening farms (β=0.29), number of confined farms (β=0.91), mean altitude (β=0.46), road density (β=0.40), proportion of open farms with at least one census (β=-0.81), human population (β=-0.38), boar population (β=-0.37), number of farms with pig movements (β=-0.31), proportion of area suitable for wild boars (β=-0.38), mean farm size (β=0.06) and farm density (β=-0.15) and certain interactions. Only the β of number of confined farms, proportion of open farms, and the interactions between mean altitude and human population and between number of confined farms and density of farms were significant ($P<0.05$).

Discussion

The proportion of open farms with at least one census in the Comune was the only protective factor. This variable may capture the amount of farms not censed (i.e. supervised by the Authorities) that are more likely to have illegal pigs. As a result, Comuni with most of their farms censed (i.e. controlled) are less likely to have ASF outbreaks. This result highlights the importance of increasing efforts not only to control the population of pigs (reducing the illegal pigs) but also to regulate the contact among pig populations. In this regard, it could be very important to increase the surveillance in the "*terre pubbliche*", which are areas that farmers can freely use to allocate and feed their animals (including pigs), and which may promote the ASFV transmission. Moreover, a large number of confined farms per Comune was a risk factor for ASFV in Sardinia. This result may be either the effect of a higher detection and/or notification of ASF in those types of farms or the consequence of a higher use of waste containing pork products to feed pigs in those confined farms, as suggested by Manneli et al. (1). Results will help to better prevent and control ASF in Sardinia and in other ASF-infected territories.

Acknowledgements

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POSSIBLE ROUTES OF ENTRY INTO FINLAND FOR AFRICAN SWINE FEVER - RISK PROFILE

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Introduction

African swine fever (ASF) is one of the most threatening diseases for pig husbandry worldwide. The disease in EU member states is currently confined to Italy (Sardinia), it was recently introduced to Caucasian regions and it is highly prevalent in sub-Saharan African countries (1). ASF has also been detected near the Northern EU border in Leningrad region (St Petersburg) in 2009 and also in January 2011, only 150 km from the Finnish border (2). Since ASF regularly occurs and re-occurs in countries around the EU, it represents a permanent and current threat for the member countries.

Materials and methods

In the risk profile different chains of events which could lead to ASF entering Finland are identified and described. Soft ticks of the genus Ornithodoros are able to transmit ASF virus. In this risk profile the role of this vector has been excluded, since there is no information available of the ticks of this genus in Finland. Data used in this profile are collected from e.g.: registries, the Customs, the Information Centre of the Ministry of Agriculture and Forestry and other experts.

Results

Potential routes for introduction of ASF into Finnish commercial pig production were several. The import of pigs to Finland is very uncommon and the pigs come mainly from Norway. The recent European Commission decision concerning the cleansing and desinfecting the livestock vehicles on arrival from Russia to EU diminishes the risk of transmitting the disease by empty animal transport vehicles, if rules are followed (3).

Small number of wild boars do cross the Eastern border frequently and thus pose a potential route for the spreading of the disease to Finland. A route to commercial production would require a direct contact between infected wild boar and pigs on Finnish pig farms. This does not seem to have great potential as a route, because wild boars have only rarely possibility to direct contact with the Finnish pig population, since outdoor farming of pigs is uncommon in Finland. Also the infected free living wild boar entering the farmed wild boar fence is not likely because of territorial behaviour and the small number of free living wild boars.

Importing contaminated goods and infected meat related to hunting of the wild boar in the countries where ASF is present is illegal. Most hunting trips from Finland are directed to Estonia.

Catering waste from means of transport operating internationally falls into category 1 (3) and is therefore handled with precautions. Animal products contaminated with ASF illegally brought into Finland by travellers, could be a matter of concern.

Discussion

This risk profile describes the probable routes of entry. Some routes seem to take place more frequently and should therefore be examined further. Illegal import of foodstuffs of animal origin for personal consumption from countries with ASF has earlier been detected to be important: in September 2009 a single outbreak was detected in the Leningrad region, following illegal movement of infectious material from the affected regions in the Caucasus (2).

Some routes enable ASF to infect the Finnish free living wild boar population. However, many quite unlikely incidents must happen before ASF in the wild boar enters the commercial pig production in Finland.

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RISK ASSESSMENT OF THE RE-EMERGENCE OF AFRICAN SWINE FEVER IN MAURITIUS.

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Introduction

Outbreaks of African Swine Fever (ASF) always have catastrophic consequences in a naïve environment, particularly in an insular locations, where stamping out all the pigs is often deemed necessary for eradication of the disease such as the case of Hispaniola in the beginning of the 80's (1). The first cases of pig mortality in Mauritius were detected in Roche Bois, close to Port Louis, in June 2007. ASF was officially confirmed in October 2007. Stamping out, biosecurity measures and movement control of pigs and pig products and closure of the abattoir were implemented (2). Stamping out was continued until March 2008; more than 10,000 pigs were slaughtered. The last outbreak was reported in July 2008 in Olivia. Assessment of the risk of re-emergence is essential to avoid another such socio-economic disaster. This assessment is also needed to fulfil the requirements of the OIE guidelines in order for Mauritius to recover its status of freedom from the disease.

Materials and methods

A first survey in November 2009 permitted the compilation of data from veterinary services, harbour and airport authorities as well as from the NPPO which is responsible for inspection and confiscation of prohibited plant and animal material. During this period field visits to evaluate farming practices were undertaken. Following stratified sampling, analyses of 603 pigs' sera for ASFV antibodies were performed using a commercial ELISA (Ingenasa). All animals tested were older than 4 months and younger than 1.5 years old to avoid any cross reaction with colostral antibodies or historical reaction. Sera of 336 domestic pigs and 12 feral pigs were analysed for presence of anti-soft tick antibodies to assess the likelihood of an existing sylvatic cycle.

Collection of data about movement in the harbour and airport as well as information about feeding habits on pig farms was completed in June 2010.

After the construction of the event model for the release and the exposure, a semi quantitative risk assessment based on the recommendation of AFSSA (3) was made.

Results

All animals tested for ASF antibodies were sero-negative. The prevalence of positive sera for anti-tick antibodies was 0.5% for Ornithodoros erraticus (only 2 positives) and 2.0% for O. moubata (7 positives in total).

The highest probability of re-introduction of the virus was found to be through the introduction of infected pig products. The particular risk presented by passengers either at the harbour or at the airport is the most important. Due to the number of passengers per year the airport is probably the most sensitive place to consider.

The probability of exposure through contaminated animal products, should the virus be introduced into the island, seems high considering the individual pig farms.

Discussion

The positive reactions for anti-tick antibodies are probably due to unspecific reactions to bites from other ectoparasites. Therefore it seems that no sylvatic cycle could occur in Mauritius. Re-emergence of ASF could only be due to re-introduction of the virus.

To control the risk passengers represent at the airport and the harbour, the use of specifically trained dogs is advised. If there is any relationship between individual pig farms and areas with high concentrations of pig farms, the spread of the disease could be dramatic. To reduce this risk, it was advised to increase the level of security at the farm level.

Acknowledgements

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DEVELOPMENT AND PRELIMINARY VALIDATION OF A PEN-SIDE TEST BASED ON THE USE OF VP72 PROTEIN FOR ASFV ANTIBODY DETECTION

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Introduction

The epidemiological situation of ASF worldwide has highlighted the need for front-line/penside tests as good tools for disease control. It will therefore assist African countries to improve their diagnostic capacity and could improve the general knowledge about the epidemiology of the disease in various regions where it is currently not possible. A lack of front-line tests also negatively impacts on routine and post outbreak surveillance. As no vaccine is available, the presence of ASFV antibodies is indicative of previous infection and, as antibodies are produced from the first week of infection and persist for long periods, they are a good marker for the diagnosis of ASF. The early appearance (from 7 to 10 days post-infection) and subsequent long-term persistence of antibodies make antibody detection techniques essential in the control of the disease. To this end, INGENASA together with the ASFRISK EU project, have developed a rapid, one-step immunochromatographic strip (pen-side test) capable of specifically detecting anti-ASF antibodies in serum specimens.

The qualitative assay is based on a direct immunoassay in which the detector reagent consisted of red super carboxyl latex micro particles covalently coated with VP72 purified protein. The capture reagent was the same protein adsorbed on the nitrocellulose membrane strip to form a test line. In addition, blue latex micro particles coated with a control protein and a second line created above the test line, by the immobilization of anti-control protein antibodies, was used as a control of test.

Materials and methods

In order to check the sensitivity and specificity of the test, a collection of serum samples previous classified as positive or negative according OIE-prescribed serological tests (ELISA and IB) has been analysed. The panel of samples have comprised; i) 70 experimental sera obtained at different days post infection from several in vivo experiments performed at URL (CISA-INIA) and IZS-UM, using the Spanish and Sardinian isolates belonging to genotype I and Kenya isolate belonging to genotype X (the most variable and genotypically distant genotype), ii) 375 field serum samples (208 typified as positive and 167 as negative by formal tests) collected in East and West endemic African countries and iii) 543 negative field serum samples from free areas of Europe.

The serum specimen was applied to the sample pad. The anti-VP72 antibodies present in the sample specifically bound to the labelled micro particles. The antibody-protein binding complex formed migrated until the nitrocellulose membrane by the flow caused by capillary action and reacted with the immobilized VP72 protein which generated a visible test line. The control latex micro particles continued to the next line, revealing a visible control line.Thus, a positive serum yielded a red test line and a blue control line, whereas a negative sample produced only a blue control line. The entire test procedure was completed in 10 minutes.

Results

For detection of ASF specific antibodies the new penside- test proved to have the same sensitivity than the OIE prescribed serological assays during the entire clinical course of the experimental infections. Slight difference was observed in one inoculated pig with Sardinia ASFV isolate in which the new assay was able to detect evidence of infection prior to OIE procedures. The diagnostic sensitivity and specificity was determined by the analyses of a total of 913 field serum samples giving rise, respectively, a 99% and 100% of sensitivity and specificity

Discussion

In summary, the results showed here allowed us to perform a preliminary validation of a single-step assay for ASF specific antibody detection which it can be performed rapidly and easily without special equipment. The immunochromatographic test provides a reliable method for detection of anti-ASF antibodies with high sensitivity, specificity and confidence where laboratory support and skilled personnel are limited. However, the current situation of ASF in Europe required performing further validation by using serum samples collected from areas affected by recent ASFV outbreaks in countries from Russia Federation and Caucasus regions.

Acknowledgements

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TWO COMMERCIAL PCR PROTOTYPE TESTS FOR RELIABLE DETECTION OF AFRICAN SWINE FEVER VIRUS (ASFV)

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Introduction

African swine fever (ASF) is a highly contagious disease of swine caused by a complex DNA virus that has been classified as the only member of the Asfarviridae family, genus Asfivirus. It produces great economic losses in the affected countries. Domestic pigs and European wild boars are very susceptible, showing a wide range of clinical forms. Epidemiological studies have demonstrated that the entrance of African swine fever virus (ASFV) in ASF-free areas is primarily related to feed pigs with contaminated garbage from international airports and seaports. This fact, together with the extensive commercial trade, puts ASF-free countries at constant risk of having the disease introduced in their territory. In the absence of vaccine, control and eradication strategies are mainly based on rapid and accurate laboratory diagnosis of ASFV-positive and carrier animals and on the enforcement of strict sanitary measures. The main object of this work was to develop ready to use one step PCR kits for the rapid and reliable detection of ASFV based on previously developed conventional and real-time PCR methods [1, 2].

Materials and methods

Conventional PCR. Optimal conditions for the PCR assay were established as follows: Primers specific for ASFV detection [1] and primers annealing within pig β-actine gene were selected to give DNA fragments different in length. After a denaturation step of 5 min at 95°C, the mix was subjected to 40 cycles of PCR, consisting of 15 s at 95°C, 30 s at 62°C, and 30 s at 72°C, and a final step for 7 min at 72ºC. Amplified DNA products were identified by 2% agarose gel electrophoresis.

Real-time PCR. A Primer set and a UPL-probe (Universal Probe Library, Roche Applied Science) specific for ASFV detection were selected [2]. A second heterologous internal control based on a specific primer-probe Texas Red-locked nucleic acid hydrolysis probe-(LNA)-system was designed for detection of a DNA fragment within the porcine β-actine gene. Optimal conditions for the duplex real-time PCR assay were established, including a preliminary denaturation step of 5 min at 95ºC followed by 45 cycles of 10 sec at 95°C, 30 s at 60°C. Products appearance was monitorized by fluorescence acquisition at the end of each cycle in FAM and Texas-Red channels.

Finally, jellification of both conventional and real-time PCR mixes was done in collaboration with Biotools.

Evaluation of the different PCR prototype kits was carried out by testing a reference collection of positive and negative ASFV DNA samples available at CISA-INIA.

Results

By using limited primer concentrations for the internal control, no adverse effects on the sensitivity of the ASF-systems could be observed, and the newly designed duplex conventional PCR or real-time PCR assays proved to have a sensitivity of approximately eight DNA copies. The selected primer-probe combination was strictly ASF-specific and no amplification was observed in non-ASFV samples tested. In order to have the possibility to use the PCR technique in less developed countries, jellification of the PCR mixes was tested to preserve them at room temperature during the transport and for storage at 4ºC.

12 jellified mixes for the conventional PCR and 5 for the real-time PCR were assayed. Specific mixes including all reagents required and providing the most robust results were selected. Finally, a one tube conventional PCR kit and a two tube real-time PCR kit prototypes were prepared, allowing the detection of ASFV on different porcine clinical material.

Discussion

In order to obtain ready to use PCR kits for ASFV detection, we have worked in the improvement of two previously described PCR methods in two ways:

On one hand, an internal endogenous control was incorporated to each conventional and real-time PCR assay to prevent the appearance of false negative results. The introduction of this control did not affect the sensitivity of any of the two PCRs. Furthermore, this internal control could be used in other pig diagnostic assays.

On the other hand, we have tested the possibility of preparing ready to use PCR kits that could be transported easily at room temperature and safely preserved at 4 ºC.

The two presented PCR prototype kits give promising results and could be of great value for rapid detection of ASFV, particularly in those regions with limited possibilities. Finally, complete validation is still required before the commercial launching of the two developed ASFV PCR kits.

Acknowledgements

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DEVELOPMENT OF CHRONIC AND ACUTE FORMS OF CLASSICAL SWINE FEVER IN PIGS EXPERIMENTALLY INFECTED WITH HIGH VIRULENCE STRAIN

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Introduction

Classical swine fever virus (CSFV) is the causative agent of one of the most devastating porcine viral diseases; classical swine fever (CSF). The virus might cause the death of animals before a specific immune response. In some cases, the immune response is not sufficient to clear the virus, leading to persistence of CSFV in the host. In endemic countries, the persistence and chronic forms are a serious problem for its control. Chronic infections can be established in the presence of neutralising antibodies and are a potential source of new CSF outbreaks (1), as well as creating problems in diagnosis. The CSFV strain Margarita has been characterized as highly virulent that induce acute clinical signs of disease. In the present work we propose mimic infection models similar to field infection caused by air, secretions and excretions transmission. Such infections are poorly characterized in terms of virus quantification; immunoresponse and their relation with the development of disease (acute and chronic). Similarly using these models we assess the ability of a highly virulent strain in the development of chronic form of CSF in experimentally infected pigs.

Materials and methods

2 boxes of NBS3 facilities of CReSA during 25 days with 17 domestic pigs (Landrace x Large white, 10 weeks old) were used. In one box, 6 domestic pigs were inoculated by intramuscular injection with 10^5 TCID of Margarita strain (group 1). Other 2 pigs were not inoculated (group 2) and were maintained in direct contact with group 1. In the second box, 6 pigs were inoculated intramuscularly with $10^{2,5}$ TCID of Margarita strain (group 3). Other 3 pigs were not inoculated (group 4) and were placed separate in the same box but in different pens (avoiding direct contact with infected pigs and their excretions). The clinical signs of disease were scored daily (from 0 to 6 points) (2) and pigs were bled at 7, 10, 13, 15, 18 and 25 days post infection to follow the specific neutralising antibody induction. E2 specific antibodies were detected using ELISA (CEDITEST). Lymphoproliferation assay were performed as described (3). The presence of CSFV RNA in serum, nasals swabs and tonsils was analyzed using the qRT-PCR real time assay (4).

Results

Groups 1, 2 and 3 developed the acute form of CSF. However, pigs from group 3 showed less clinical signs than groups 1 and 2. In contrast, group 4 (indirect contact) developed a mild form of disease from 11 to 25 days post infection (dpi) (clinical score of 0.3 to 3). Only pigs from group 4 were positive in the Lymphoproliferation assay against virus and concanavalin A stimulus at 7, 10, 15, 18 and 25 dpi. One pig however showed neutralizing antibodies response and E2 specific antibodies at 25 dpi. The RNA viral load was detected at 7 dpi only in groups 1 and 3 in serum and oropharyngeal swabs samples in high concentration. At 10 dpi the RNA viral load was detected in serum and oropharyngeal swabs samples in all pigs tested form 1, 2 and 3 groups. On the contrary in group 4 only the RNA viral load was detected in oropharyngeal swabs samples at 10 dpi. Likewise, at 15, 18 and 25 dpi, the RNA viral load was low in serum and oropharyngeal swabs samples tested from group 4. Finally, RNA viral load was similar (high concentration) in all the tonsil tissue samples tested from all groups.

Discussion

In the present study, we developed two experimental infection models that mimic the acute and "sub-lethal-chronic" CSF form using a high virulent strain. The pigs from group 4 developed the sub lethal-chronic CSF infection based on clinical, immunological and virological parameters. The forms of CSF obtained were directly associated with the viral dose and the route of inoculation. It is previously reported that high virulent strain usually spreads rapidly throughout the body, resulting in high virus titres in most organs and blood, whereas with moderately virulent strains, virus titres tend to be lower (5). Our results suggest that secretions and excretions from pigs of group 3 (infected with high virulent strain) may be responsible for virus transmission between groups 3 and 4. On the other hand, also it suggests the involvement of highly virulent strains in the induction of chronic forms of infection. In addition, the possible co-circulation of strains with different degrees of virulence in endemic countries and control measures that reduce viral load may be involved in the evolution of CSFV in the field, and in the development of chronic and persistence forms (6).

Acknowledgements

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CLASSICAL SWINE FEVER SEROPREVALENCE RESULTS IN WILD BOARS IN YEAR ONE (2009) AND TWO (2010) AFTER AN OUTBREAK

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Introduction

The wild boar has been recognised as the main reservoir of classical swine fever (CSF) virus in Europe (1). In Croatia, the last detection of CSF virus in wild boars was in 2008 in Sisak Moslavina County (SMC) and Vukovar Srijem County (VSC) when control measures and monitoring have been applied. This study presents our results of a thorough surveillance program regarding the County of origin, sex and age of wild boars investigated.

Materials and methods

During 2009, 4857 samples originating from wild boars have been tested at the Croatian Veterinary Institute for the presence of CSF virus and antibodies (Table 1). Among these, 1233 samples originated from wild boars from the SMC and 683 from the VSC (Table 2). In 2010, 8759 samples of wild boars were tested (Table 1) of which 2564 were collected in SMC and 902 in VSC (Table 2). Samples were tested by commercially available ELISA kits (PrioCHECK CSFV 2.0 Ab, Prionics) and QRT-PCR (Adiavet CSF Realtime PCR kit, Adiagene).

Results

All samples tested gave negative results for virus detection. Our results show a statistically significant ($p<0.001$) elevated appearance of antibody prevalence in female animals compared to male animals in both investigated periods. Most of the positive wild boars in 2009 were older than 2 years of age; however, positive animals of one to two years of age were shown to be statistically significantly more frequent than positive animals up to one year of age ($p<0.001$). In 2010 there was no statistically significant difference recorded between the appearance of seropositive animals up to one year of age and from one to two years of age. Only an increase in seroprevalence was detected when compared to wild boars older than two years of age (Table 1). During 2009 there was a statistically significant ($p<0.001$) elevated prevalence of CSF antibodies in the SMC in comparison to the VSC, which was not the case in 2010 (Table 2).

Discussion

In contrary to our previous studies (2) the newly gained results show that the majority of the positive animals found after a recorded outbreak were female animals older than 2 years of age, therefore this could be the most suitable age category for further monitoring programs. Even though the same measures have been applied in both investigated Counties, a significant decrease since 2008 in the seroprevalence has been recorded only in VSC. This could be a result of more efficient control measures in an area where the hunting ground is fenced in comparison with the open hunting ground in the SMC. Therefore, additional measures, as oral vaccination accompanied by a surveillance program would be recommended for the control of CSF in the SMC.

Table 1. Distribution of positive wild boar samples according to sex and age.

2009		
Sex	Age	Tested/positive /% of positive
Female	< 1 year	1298/5/0.38
	1-2 years	342/12/3.5
	> 2 years	199/20/10.5
Male	< 1 year	1467/5/0.34
	1-2 years	686/15/2.18
	> 2 years	445/3/0.67
No data		420/3/0.71
2010		
Female	< 1 year	2217/10/0.45
	1-2 years	842/6/0.71
	> 2 years	669/20/2.98
Male	< 1 year	2521/6/0.23
	1-2 years	1217/2/0.16
	> 2 years	909/24/2.64
No data		384/0/0

Table 2. Number of tested/positive wild boar samples according to Counties and investigation period.

	SMC	VSC
Year	Tested/positive	Tested/positive
2009	1233/27	683/37
2010	2564/63	902/15
Total	4844/97	2219/170

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AFRICAN SWINE FEVER SURVEILLANCE IN NIGERIA, 2008 - 2009.

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Introduction

African Swine Fever (ASF), a transboundary animal disease, is a viral disease of pigs. It is prevalent in sub-saharan African countries. ASF is a notifiable disease as classified by the World Organization for Animal Health (OIE) and is caused by a DNA virus of the family Asfaviridae and genus Asfivirus [1,2]. ASF is a disease with no treatment or vaccine available therefore control relies on rapid laboratory diagnosis and implementing hygienic measures. In 1997, outbreaks of ASF associated with high mortality were first reported in Nigeria causing significant socio-economic impact on people's livelihood. Surveillance of ASF was carried out between October 2008 and December 2009 to determine the prevalence of infection in the pig population.

Materials and methods

Using cluster sampling for the six geo-political zones, two states were randomly selected. Pig farms, markets and abattoirs were selected from each cluster. Structured questionnaires were used to obtain data on outbreaks and handling of sick animals from farmers. Blood was collected from apparently healthy pigs and tissue samples from slaughtered animals and tested for ASF by ELISA, PCR and virus isolation.

Results

Of 1,274 serum-samples analyzed, 111 (8.7%) tested positive for ASF. Of the 330 tissue-samples, viral DNA were detected in 157 (47.6%) samples, out of which 77 (49.0%) virus isolates were obtained. Of the 39 farmers interviewed, 30 (76.9%) experienced ASF outbreaks with a total of 2,712 pig deaths. Fifteen (50.0%) farms recorded mortality rates of over 50%. Out of 30 farms, 19 (63.3%) had other farms with outbreaks in their neighborhood, 25 (83.3%) sold sick pigs on their farms, while 7 (23.3%) disposed carcasses carelessly.

Table 1. Summary of the 2008/2009 African Swine Fever Surveillance

Region	Serology (I-ELISA)			Virology (PCR)		
	Total	Positive	%	Total	Positive	%
S/West	225	14	6	57	41	72
S/South	194	23	12	56	13	23
S/East	69	3	4	23	0	0
N/West	74	2	3	19	16	84
N/East	189	28	15	3	0	0
N/Central	523	41	8	172	87	51

Discussion

ASF virus is still circulating in pig population in Nigeria with frequent outbreaks. It is evident that our surveillance method had some limitations since it was carried out in selected locations hence some regions may have been under represented based on the estimated pig population verbally communicated by stakeholders. The surveillance was done in targeted areas with high pig population.

The practices of farmers might have an influence on spread of the disease[3]. Some farmers expressed dissatisfaction with the government and did not allow sampling in their farms since no compensation was paid for pigs that had died due to ASF in past outbreaks. Farmers need to be sensitized on Biosecurity measures taken during ASF outbreaks to ensure that the disease is contained only in affected farms.

Acknowledgements

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EPIDEMIOLOGICAL SURVEY ON SEROPOSITIVITY FOR CLASSICAL SWINE FEVER, PRRS, SWINE VESICULAR DISEASE AND AUJESZKY'S DISEASE IN HERDS IN NORTHWEST ITALY

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Introduction

The aim of this study is to evaluate the seropositivity for Classical Swine Fever, Swine Vesicular Disease, PRRS and Aujeszky's Disease in a sample of swine herds located in Pinerolo district, northwest Italy. These disease represent a significant risk in pig farms, especially in areas with a widespread livestock. From 1997 to 2010 , a sample of sows and fattening pigs selected randomly were tested to research antibodies against these diseases.

Materials and methods

In Italy, exist a mandatory program to the control and eradication of Classical Swine Fever and Swine Vesicular Disease from 1994 and for Aujeszky ' s Disease from May 1997. We tested 8415 pigs for Swine Vesicular Disease, 7306 for Aujeszky's Disease, 4860 for Classical Swine Fever and 816 for PRRS in 802 herd's visit. Serum samples collected were tested for antibodies by the enzyme-linked immusorbent assay (Elisa test).

All farrow to feeder herds was tested twice' s year while fattening herds were tested for a sample. We collected data on the results of serological tests and evaluated disease prevalence.

Results

Classical Swine Fever: all sows and fattening pigs tested were negative.

Swine Vesicular Disease: all were negative, except 25 sows that resulted positive in a single farm in 2002. All positive sows did not show clinical signs.

Aujeszky's Disease: The seropositivity prevalence in tested pigs decreased from 38 % in 1997 to 3,9 % in 2010. The seropositivity herd's prevalence decreased from 84 % in 1997 to 2 % in 2010. In the years from 2005 to 2010 the average seropositivity prevalence in tested pigs was 4,33 %.

Porcine Reproductive Respiratory Syndrome (PRRS): the results show that antibodies were present in a great number of sows (79 %). The seropositive farms resulted 94 % of total. Many farms showed significant economic losses due to disease.

Discussion

The present study shows that Classical Swine Fever and Swine Vesicular Disease are eradicated in farms tested. However, our results indicated that Aujeszky's disease and PRRS are still present. This study underlines the diffusion of the PRRS in our herds, in fact this disease shows a high seroprevalence in many farms causing serious health problems and severe economic damage (4,5,6,7). This research proves also that epidemiological status for Aujeszky' s disease is in marked improvement and that biosecurity measures associated with compulsory vaccination are an important instrument (1,2,3).

Eradication of Aujeszky's disease and control of PRRS are a primary objective in the coming years.

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RAPID DETECTION OF CLASSICAL SWINE FEVER USING A NEW TARGET REGION - THE REAL-TIME RT-PCR TEST KIT VIROTYPE CSFV

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Introduction

Classical Swine Fever (CSF), an internationally notifiable animal disease, is economically one of the most important viral infectious diseases of swine. CSF is prevalent worldwide in pigs and wild boars and endemic in wild boar populations of several EU member states. The causative agent Classical Swine Fever Virus (CSFV) is a single-stranded RNA virus, a member of the genus Pestivirus which belongs to the *Flaviviridae* family and is closely related to bovine viral diarrhoea virus (BVDV) in cattle and border disease virus (BDV) in sheep. Real-time reverse transcription polymerase chain reaction (real-time RT-PCR) is a sensitive and specific method for detection of CSFV. Previously published protocols use the highly conserved 5' untranslated region (5'UTR) of the CSFV genome as template for CSFV diagnosis. In order to ensure highest safety in molecular diagnostics of CSFV a new real-time RT-PCR with a target region independent of the 5'UTR was developed.

Materials and methods

Test material was CSFV RNA from swine blood and tissue samples. The samples were tested with VIROTYPE® CSFV in comparison to an in-house CSF-1 method according to Hoffmann et al. (1). VIROTYPE® CSFV includes all reagents for the detection of CSFV RNA as well as a positive and a negative control. This new CSFV system is duplexed with a β-actin detection system as internal control in the same tube. This allows the control of extraction as well as amplification. Due to the high sensitivity of the test - which is approved by the Friedrich-Loeffler-Institut - pools of up to 20 samples may be analysed. The test protocol is a simple one-step procedure of adding RNA samples directly to the ready-to-use CSFV- Mix.

Results

High analytical sensitivity of the VIROTYPE® CSFV kit was proven by a titration series of CSF in vitro RNA. The VIROTYPE® CSFV is able to detect at least 10 copies per sample. For validation purposes different relevant European CSFV genotypes and a number of recent European CSFV field isolates were tested and reliably detected. RNA samples from CSFV-positive tissue (spleen, kidney, tonsils and lymph node) provided by the German National Reference Laboratory for CSF and of plasma, serum and leucocytes provided by the EU Community Reference Laboratory for CSF were detected more sensitive than with in-house-RT-PCR. Wild boar blood samples could reliably be detected with VIROTYPE® CSFV at a German State Diagnostic Laboratory in comparison to the established in-house method. High specificity of the VIROTYPE® CSFV was demonstrated by testing RNA samples from different genotypes of related Pestiviruses (e. g. BVDV-1, BVDV-2 and BDV) and of persistently infected BVDV positive calves. No cross reactivity was observed with these samples.

Discussion

In summary, the new VIROTYPE® CSFV is a robust, highly sensitive and fast test kit which allows a reliable detection of CSFV genome independent from the 5'UTR genome region. Because of the easy to use test protocol the test kit is suitable for high throughput testing and is also valuable as an independent confirmatory diagnostic method.

Acknowledgements

This work was realized in scientific collaboration with Dr. Bernd Hoffmann and Dr. Martin Beer of the Federal Research Institut for Animal Health, Friedrich-Loeffler-Institut, and supported by Sächsische Aufbaubank (SAB).

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EFFECTIVENESS OF THE C-STRAIN VACCINE FOR RAPID PREVENTION OF CLASSICAL SWINE FEVER TRANSMISSION

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Introduction

The live, attenuated C-strain vaccine provides effective immunity against classical swine fever virus (CSFV). However, within the EC, its use is limited to emergency outbreak situations (1) because of an inability to distinguish vaccinated from infected animals using serology. Our study sought to determine the interval required for the C-strain vaccine to induce protective immunity and therefore whether it could be deployed as a control measure during an outbreak. We also investigated antibody, T cell and cytokine responses as potential correlates of protection associated with vaccination to inform the design of future vaccines.

Materials and methods

In two separate experiments, pigs were mock-vaccinated or vaccinated intramuscularly with the Riemser C-strain genotype 1 vaccine at 1, 3 or 5 days prior to challenge. Pigs were then challenged by intranasal inoculation with the genotype 2 strain UK2000/7.1, that caused the most recent outbreak in the UK (2) or the genotype 3 strain CBR/93 isolated in Thailand (3). In-contact animals were introduced 24h post-challenge. Animals were assessed for clinical signs and samples were obtained at 2-3 day intervals to monitor viraemia, leukopenia, cytokine responses and antibody production. Peripheral blood cells were isolated, stimulated with CSFV and IFN-γ release into culture supernatants was assessed by ELISA. When the humane endpoint was reached, or when the experiment was terminated, animals were necropsied. Histological and cytokine analysis of tissues was conducted.

Results

Inoculation of mock-vaccinated animals with both the UK2000/7.1 and CBR/93 strains resulted in the development of clinical signs, marked leukopenia and high viral loads in circulating leukocytes. When animals were vaccinated and then challenged after 5 days, all parameters measured indicated that they were protected from disease and infection was not transmitted to in-contact animals. Animals challenged 1 day post-vaccination were positive for all assays and transmitted infection to in-contact animals. Vaccination of animals 3 days prior to challenge provided partial protection from clinical disease, but transmission of virus to in-contact animals occurred in one experiment. Neutralizing antibody responses in protected animals were detected beginning at 9 days post-challenge, whereas this response was delayed in animals where replication of challenge virus could be detected. *In vitro* stimulation of leukocytes with CSFV revealed that cells isolated from animals vaccinated 5 days prior to challenge produced a strong IFN-γ response, whereas the response elicited in cells isolated from animals vaccinated 1 and 3 days prior to challenge was delayed and weaker. Cells isolated from mock-vaccinated, inoculated animals failed to mount an IFN-γ response upon stimulation. Similarly, IFN-γ levels were elevated in tissues from vaccinated, protected animals.

Discussion

These data show that C-strain vaccination effectively prevents infection and transmission of genetically diverse strains of CSFV within 5 days. Vaccination 1 and 3 days before infection provides some protection to vaccinated animals but does not block virus transmission to mock-vaccinated, in-contact animals. The rapid protection afforded by C-strain vaccination most likely depends on appropriate induction of innate immune mechanisms and IFN-γ is a good correlate of this protection.

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EFECTIVE HUMORAL RESPONSE TO E2 SUB-UNIT MARKER VACCINE CANDIDATE AGAINST CSFV IN NATURALLY INFECTED ANIMALS WITH PCV-2

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Introduction

Classical swine fever virus (CSFV) produces a highly contagious and often fatal multisystemic disease in swine with a negative impact concerning both economic and sanitary issues. In Cuba, the evaluation of the response to CSFV vaccines in pigs affected by PCV2 is very important because both viral agents have been reported simultaneously in the same samples from the swine herds examined (7). On the other hand, immunological impairment by porcine circovirus type 2 (PCV2) infection is well documented in pigs (1, 2). Several lines of evidence suggest that the immunosuppressive activity of PCV2 may confer a negative impact in swine affected by other diseases (1, 2). Recently we have obtain a new E2 sub-unit marker vaccine candidate against CSFV that confers solid protection in pre-clinical trials. The aim of this work was to evaluate the effective humoral response to E2 sub-unit marker vaccine candidate against CSFV in naturally infected animals with PCV2.

Materials and methods

Three litters at 3 weeks old were detected in the same week with clinical signs of circovirus infection. The herd had been vaccinated 5 months ago with a protein subunit vaccine (glycoprotein E2) (8) vs. CSF. The piglets received two doses of vaccine, at 21 days after birth and at 42 days old. Their sows had been vaccinated in the previous weaning. Blood samples were taken after 7 days of the first vaccination dose in piglets and their mothers for PCV2 diagnosis by PCR. Sera were evaluated for CSF by NPLA at T0 (28 day), and five piglets of each litter at 42, 70, 140, 168 and 196 days. Animals were tested too by ELISA in the first three sampling (T0, 1 and 2) and by Direct Immunoperoxidase (DIP) with monoclonal antibodies in tonsils taken at their slaughter. The three litters affected were maintained in separate pen after weaning (33 days) until the beginning of fattening period when all of them were regrouped in the same pen.

Results

PCV2 was previously diagnosed in the herd in pigs with clinical signs and lesions compatible with Porcine Dermatitis and Nephritis Syndrome (PDNS). Also, there were frequently many pigs with wasting and pale skin named “white pigs”. At weaning, the litters of sows A, B and C had 11, 10 and 11 piglets, respectively. Only 3 piglets of the sow A have a normal status, the others are with moderated health status. The sows B and C had had a bad reproductive performance before. PCV2 infection was confirmed in piglets of all litters and the sow A and B. The sow C (No tested) could be infected, because its piglets were also infected. Sixteen pigs were positive to PCV2 (16/18, 88.9 %). The mortality of the group was high (9/32 death, 28.1 %). Their weight was less than media (85 kg) at the slaughtering. Titters began to decrease at 140 days, but all pigs tested in the slaughterhouse at the end of the fattening period showed titters at least =1:100 and were DIP negative.

Discussion

In spite of the PCV2 infection when almost all piglets were positive to DNA viral detection, the pigs showed a satisfactory humoral response to E2 subunit vaccine measured by NPLA and ELISA tests. When titers of at least 1:100 were observed, that correlated with protection against CSFV replication. In addition, all pigs from litters assessed were negative at RNA CSFV detection. Nevertheless, further studies to clarify the possibility of CSF vaccination failure in herds affected by PCV2 in under active investigation.

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CLASSICAL SWINE FEVER WORKING GROUP OF CARIBVET NETWORK: EXAMPLE OF A REGIONAL APPROACH TO MONITOR AND CONTROL SWINE DISEASES IN THE CARIBBEAN

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Introduction

Classical swine fever (CSF) was first introduced in the Caribbean (Cuba) in 1930. After 25 years of absence, the disease re-emerged in 1993 in Cuba, then in Haiti (1996) and Dominican Republic (DR) (1997) causing sanitary crisis. In these countries, pigs are mainly maintained in backyards, but there is also an important industrial swine population in Cuba and DR. The high cost of control programs are an important issue for the effectiveness and sustainability of such programs in the infected countries.

This initiated a regional approach of surveillance and control. The Caribbean Animal Health Network, CaribVET (1), is a regional collaborative network involving veterinary services, laboratories, research institutes from all countries - territories of the Caribbean and regional - international organizations. Its objective is to improve animal health situation in the Caribbean. The CSF working group, one of the six CaribVET technical working groups, was created in 2008. It addresses CSF issues related in the region.

Materials and methods

The CSF working group gathers twenty experts in swine diseases and veterinary services from infected countries or countries considered at risk because lot of exchanges of humans, animals and animals products in the Caribbean. It meets once a year and its objectives are to provide a regional expertise on swine diseases, to work out harmonized surveillance and control protocols and strategies, to improve diagnosis capacities in the region and to define regional emergency plans. At the end of each meeting, the CSF working group makes recommendations to determine future regional or national activities and work strategies.

Results

CSF working group members work on harmonized surveillance and control plans and on the implementation in their countries.

Annually, laboratories of Haiti, DR and Cuba participate in Interlaboratory Assays with the EU/world OIE-CSF reference laboratory of Hannover (Germany) in order to assess and improve their diagnosis capacities.

Veterinary services and laboratory staff from the Caribbean are regularly trained to conduct epidemiological studies and to improve preparedness in case of disease outbreak.

CSF working group members participated in several international congresses to give updates of the CSF situation in Caribbean countries and to promote their activities.

The working group developed a conceptual framework based on the Cuban risk analysis manual (2) to evaluate the risk transmission of CSF between countries. This framework was validated by the Epidemiology working group of CaribVET and conceived as a pragmatic tool to help countries to strengthen their surveillance systems to mitigate risks.

The group designed questionnaires to assess the humans, animals and animal products traffic among Caribbean countries to identify places at risk of CSF introduction where high level of surveillance should be maintain.

Discussion

Though CSF is the subject of control programs in the three affected countries, this disease is still not eradicated from the Caribbean. Moreover, other important swine diseases circulate in the region: Circovirus (3), Torque Teno Sus virus and, since 2009, Teschen encephalomyelitis in Hispaniola with major economical and social impacts. So, the CSF working group, in agreement with CaribVET steering committee, decided to expand its activities to swine diseases and adapt its activities with the sanitary situation evolution.

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CHIMERIC CALICIVIRUS-LIKE PARTICLES ELICIT IMMUNE RESPONSES AGAINST A T-CELL EPITOPE OF FOOT-AND-MOUTH DISEASE VIRUS IN PIGS

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Introduction

Virus-like particles (VLPs) are viral subunit vaccines that have unique advantages in terms of safety and immunogenicity (1). VLPs from rabbit hemorrhagic disease virus (RHDV) have shown to be good foreign epitope carriers for vaccine development in mice (2).

Foot-and-mouth disease (FMD) is the most economically important veterinary disease on the condition and productivity of the many animal species it affects (3).

For this study, we generated chimeric RHDV-VLPs, produced in insect cells (4), containing a T epitope of FMDV 3A protein (5) in the N-terminal part. The aim of this work was to assess whether RHDV-3AT-VLPs were able to exert some immunogenicity in pigs, a major natural FMDV host.

Materials and methods

Fifty conventional pigs, 6-7 weeks old, were divided in 3 groups of 15 animals (B, C, D) and one control group (A) of 5 animals. Groups B, C, D were inoculated twice in 2 week interval with chimeric RHDV-3AT-VLP at different doses and using different routes of inoculation (**Table 1**). Additionally, group C was inoculated intramuscularly with adjuvant (ADJ) Montanide™ ISA 206 (SEPPIC).

Serum, blood with EDTA, swabs and saliva were collected at day 14 and 28 after the first immunisation. Peripheral blood mononuclear cells (PBMCs) were obtained and analyzed for specific IFN-γ production by ELISPOT and for lymphoproliferation assay by [*methyl* -^{3}H]thymidine incorporation. After culling at D28, IM inoculation site was collected for histopathological studies.

Table 1.Experimental design: pigs inoculated with different routes and different doses (μg/pig). IN: intranasal; IM: intramuscular; ADJ: adjuvant.

Group	Route	μg/pig
A	-	-
B	IN	20 /60 /180
C	IM+ADJ	20 /60 /180
D	IM	20 /60 /180

Results

Two weeks after the last inoculation with RHDV-3AT-VLPs, specific IFN-γ-secreting cells against 3AT were detected in PBMCs by ELISPOT. Pigs of group C have shown the highest number of IFN-γ-secreting cells compared with the other groups. As expected, control pigs did not show any significant response.

Induction of FMDV-specific T cells was also detected in lymphoproliferation assay with PBMCs of RHDV-3AT-VLP immunized pigs. High specific responses against VP60 (capsid protein of VLPs) and 3AT were found in group C not only after the second inoculation but also after the first inoculation. No stimulation was observed in control pigs.

Lesion score at the inoculation site was higher in group C than in the other groups. Group D lesion score was comparable to control pigs.

Discussion

In this study we generated chimeric RHDV-VLPs carring the T epitope of FMDV 3A protein, which have shown a powerful capacity to elicit a potent anti-viral response in pigs. Pigs were able to elicit specific IFN-γ-secreting cells against the FMDV T epitope 14 days after the second intramuscular dose of RHDV-3AT-VLPs with adjuvant, showing the high immunogenicity potential of these constructs.

This is the first immunological report on the potential use of chimeric RHDV-VLPs as antigen vectors in pigs.
Future studies have to be performed to investigate the capacity of these particles to protect pigs against FMDV.

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WHAT WOULD HAPPEN IF A FINNISH PIG FARM WOULD HAVE A FMD-OUTBREAK?

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Introduction

Finland does not have recent experience in FMD epidemics since the last outbreak of FMD appeared 1959. However, estimates for the magnitude and consequences for a possible FMD outbreak are required for contingency planning and maintenance of preparedness. The aim was to study the spread of FMD within a country with relatively sparse production structure.

Pig meat production is over two times larger than cattle meat production in Finland (1) but the number of farms is lower: only 10% of animal production farms operate in pig production sector (n=3225). Pig farms operate typically only in one sector as only 7% of farms have also other animals. Pig production is concentrated into two regions: Western and South-western parts of the country (together 66% of pig farms), while other livestock production sectors are more widely scattered (2).

Materials and methods

The spread of FMD in the country was simulated by a model which simulates different contact types from an infected farm(s) during an infective period. The basic approach of the model is similar as in models like Interspread and InterCSF (3, 4). Parameterisation and operation of the model is described in details in the recent Finnish FMD risk assessment report (2).

Results of this study are based on a simulation (n=14039), where outbreaks were simulated to start from each Finnish pig farm.

Results

If a FMD would be introduced into Finnish pig farm it would remain small: typically it would contain only 5 infected farms (97% <18 infected farms). The probability that the outbreak would spread beyond the pig production sector increases together with the final epidemic size, but even in the largest outbreaks (>18 infected farms), 51-76% of the infected farms would be pig farms. In addition, large part of uninfected farms in restrictive zones would operate in pig production sector. Regional differences were apparent: in pig farm dense South-western region 46% of uninfected farms in restrictive zones would be pig farms but on the contrary on the other parts of the country corresponding proportion would be only 26-32%.

Discussion

Expected final epidemic size would be small in Finland which indicates that a FMD outbreak could be controlled adequately by standard EU control measures

When the primary infected farm would be a pig farm, also most of the infected and large part of uninfected farms situated in restriction zones would be pig farms. This is partly because of the concentrated nature of the pig production, where most of connections are occurring within the production sector.

Regional differences in the number of uninfected farms in administrative zones were large and should be taken into account when regional contingency plans are updated.

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EVIDENCE FOR PORCINE PARVOVIRUS TYPE 4 (PPV4) IN BRAZILIAN SWINE HERDS

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Introduction

Porcine *bocaviruses* were recently identified among swine co-infected with PCV2 (2, 3) and suffering an acute-onset disease of high mortality in the United States, in pigs with PMWS in Sweden (1), and in pigs with reproductive and neurological disease in China (4).

Parvoviruses are small and non-enveloped icosahedral viruses. According to the ICTV, the *Parvovirinae* subfamily is classified into five genera: *Dependovirus, Bocavirus, Erythrovirus, Parvovirus* and *Amdovirus*. Based on new virus discoveries, two new genera, *Hokovirus* and *Cnvirus*, have been proposed. In addition, there are two bovine isolates (BPV2 and BPV3) and one porcine isolate (PPV4, a *bocavirus*) that do not cluster with any member of the *Parvovirinae* subfamily (3, 5, 7).

The PPV4 genome contains two major open reading frames (ORF1 and ORF2), coding for the non-structural protein, located at the 5'-end, and the capsid protein, located at the 3'-end (3, 5). An additional ORF3 has been observed among viruses of the genus *Bocavirus*, including PPV4. ORF3 is located in the middle of the viral genome, between ORF1 and ORF2, and is essential for viral DNA replication (5, 7).

PPV, PCV2, TTV and other single stranded DNA viruses have been detected and associated with reproductive failure (6). The objective of this study was to detect and sequence the genome of PPV4 isolates from reproductive organs collected from culled sows, aiming to verify the presence of PPV4 at these sites.

Materials and methods

The ovaries and uteri from 83 sows were collected at a slaughterhouse in Santa Catarina state, Brazil, between July 2008 and July 2009. Viral DNA was extracted from a pool of uterus and ovary tissues of each sow with a standard phenol:chloroform:isoamyl alcohol extraction procedure. The PCR reaction was carried out using specific primers (F5'-TAT GTG GGC TGG GCA AGG AAT GTC-3') and (R5'-GTT GCG GAA TGC TAT CAG GCT CTT-3') designed for ORF3, targeting a 440 bp amplicon. The reaction mixture was heated at 95°C for 5 min, amplified for 35 cycles at 95°C for 45 s, 63°C for 1 min, and 72°C for 1 min, and then kept at 4°C. The PCR products were gel-purified using BigDye XTerminator Purification Kit (Qiagen) and nucleotide sequences were determined using an AB-3130*xl* Genetic Analyzer. Sequences obtained were analyzed using BLASTx and were aligned using Bioedit Sequence Alignment Editor and Clone Manager 7

Results

Thirty-five out of 83 samples (42%) tested positive for PPV4, with a DNA amplicon of the expected size clearly detectable. Ten out of 35 positive samples were electrophoresed in an agarose gel, and three were purified and sequenced. Two out of three samples were aligned with results showing 99% and 100% identity with the other nine PPV4 submissions available in GenBank. A single synonymous nucleotide change in a conserved region was found (T to C) in one sample, thus encoding the same amino acid.

Discussion

Our findings represent the first identification of PPV4 in Brazilian herds. PPV4 has been described in USA (3) and in China (4), and appears to be ubiquitous. PPV4 ORF1 is 1,794 nt long and capable of encoding a protein of 588 residues, PPV4 ORF2 is 2,184 nt long and capable of encoding a protein of 728 residues, and ORF3 is 612 nt long and capable of encoding a protein of 204 residues. Whereas PPV4 is most closely related to BPV2, with respect to ORF1 and ORF2, the coding capacity and genome organization of PPV4 (ORF3), are more similar to those of viruses of the genus *Bocavirus* (3, 4, 7). In conclusion, the results of this study demonstrate that PPV4 exists in pig farms in Brazil, probably since 2008. Cloning of PPV4 followed by whole genome sequencing is being carried out. The prevalence, epidemiology and genetic diversity of PPV4 are currently being investigated to try to understand the significance of PPV4 within the swine population.

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PORCINE PARVOVIRUS INFECTIONS IN WILD BOAR POPULATIONS OF ROMANIA

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Introduction

Porcine parvovirus 1 (PPV1) belongs to the genus *Parvovirus* of the *Parvoviridae* family, and is responsible for serious reproductive disorders in unprotected sows. A tentative member of the family PPV3 or porcine Hokovirus (PHoV) was recently discovered in Hong Kong (4) and is closely related to the human parvoviruses 4 and 5 (PARV4/5) and bovine Hokovirus (BHoV, 2). Little information is known about the presence and prevalence of PHoV worldwide, but a study in Germany showed high prevalence of PHoV - 32.7% (1) in wild boars. Another new parvovirus (PPV4) was identified in lung lavage of a diseased pig coinfected with PCV2 (2) indicating that perhaps PPVs were more diverse than earlier believed. The aim of this study was a spatio-temporal analysis of PPV infections in wild boar populations of the Western region (Transylvania) of Romania.

Materials and methods

Samples (spleen, liver, lung, kidney, lymph nodes, tonsils) of wild boars from 315 different hunting grounds from Transylvanian counties were collected during the 2006/2007 (n=470) and 2010/2011 (n=372) hunting seasons (Table 1). Viral nucleic acid was extracted from homogenized tissues using the Viral DNA/RNA Extraction kit (Viral Gene-spin TM, Intron Biotechnology, Inc., Korea). Short PPV sequences were amplified by PCR using published primers, or primers designed according to GenBank sequences (2,3,4) and detected by agarose gel electrophoresis.

Results

The studied PPVs were all present in the samples but the prevalence of infections was different among hunting seasons (Table 2). PPV1 was detected in 10 animals during the 2006/2007 hunting season (2.12%) while the samples collected in 2010/2011 showed a higher value (9.13%). A remarkable difference was observed in case of PHoV, where the prevalence of 2006/2007 (22.76%) increased to 47.04% in 2010/2011. Only 4 PPV4 sequences were detected for both hunting seasons.

Discussion

The results showed that the PPV1 and the newly discovered PHoV and PPV4 were present in Transylvanian wild boar populations. This is the first description of the presence of PPV4 in wild boars. While the prevalence of PPV1 and PHoV seems to be increasing, PPV4 remained at a very small percentage. The clinical and pathological implications of the presence of the new PPVs are not known yet.

Table 1. Distribution of hunting grounds and wild boar samples from Transylvania.

Counties	Hunting grounds	2006/2007	2010/2011
Satu Mare	26	43	32
Bihor	37	29	39
Arad	25	23	15
Timisoara	4	6	35
Caras Severin	19	0	23
Alba	21	29	12
Hunedoara	13	33	12
Cluj	23	28	31
Salaj	9	3	17
Maramures	14	11	11
Bistrita Nasaud	14	8	30
Sibiu	30	102	19
Brasov	10	10	1
Harghita	21	51	28
Covasna	17	62	33
Mures	32	32	34
Total	**315**	**470**	**372**

Table 2. Prevalence of PPVs in Transylvanian counties during two hunting seasons.

Virus	Hunting seasons 2006/2007	2010/2011
PPV1	10/470 (2.12%)	34/372 (9.13%)
PPV3	107/470 (22.76%)	175/372 (47.04%)
PPV4	4/470 (0.85%)	4/372 (1.07%)

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PREVALENCE OF PORCINE PARVOVIRUSES IN CIRCOVIRUS INFECTED HERDS

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Introduction

Porcine parvoviruses (PPV) are present worldwide and up to recently they were believed to form a single serotype (PPV1), the one responsible for reproductive disorders. Reports from the last years however, indicated that besides PPV1 other parvoviruses were also present in pigs. The pathogenic role and the worldwide distribution of the newly identified viruses are not clear yet. PPV2 was detected in 2001 in Myanmar (3) and has not occurred since. PPV3 or HoKo virus was first identified in Hong Kong (4) and reports suggest a worldwide presence of the infection, with unknown clinical consequences. PPV4 (2) is most closely related to bovine parvovirus 2 but also shares genomic features with bocaviruses. The objective of this study was a retrospective investigation of the presence of PPVs in Hungarian pig farms infected with porcine circoviruses (PCV).

Materials and methods

Organ samples from 322 domestic pigs of 63 swine herds distributed throughout the country were collected between 2005 and 2010. A number of farms were sampled yearly. The samples were processed for DNA extraction (NucleoSpin® Tissue Kit, Macherey-Nagel GmbH & Co., Germany) and tested for the presence of PPV1, 3, 4 and PCV2 using published protocols (5, 1) or primers designed based on available sequences (4, 2). Amplicons were detected by agarose gel electrophoresis according to standard protocols and selected products were sequenced for further analysis. The sequences were compared with each other and with those available in the GenBank.

Results

Besides the wide distribution of PCV2 all of the tested porcine parvoviruses were present in the samples, although in different proportions (Table 1). PPV1 was detected in 4 animals of 3 farms, and sequencing showed that they were different from the generally used vaccine strains. PPV4 was detected in samples from 2006 to 2009 in 23 pig farms. PPV3 was present in the highest proportion of the samples, and according to the PCR results the occurrence of the virus was increasing throughout the years.

Table 1. The incidence of PCV2 and PPV.

year	farm / sample	PCV2	PPV1	PPV3	PPV4
2005	1/1	1/1	0/0	1/1	0/0
2006	6/64	6/64	0/0	1/1	2/3
2007	32/112	32/112	0/0	11/19	13/16
2008	25/92	25/92	2/3	15/42	7/9
2009	17/43	17/42	1/1	14/28	1/2
2010	1/10	1/5	0/0	1/8	0/0
total		63/316	3/4	37/99	20/30

Results are indicated as positive farms / positive samples.

Discussion

The survey confirmed the previous data that PCV2 was widespread in Hungary, as it is in other areas of the world. The PCV2 detected in this study was all of the PCV2b type. The wide variety of PPV types present however, was not known. Vaccination against PPV1 is used widely and the presence of wild type infection in vaccinated animals raises concerns of efficacy in herds infected with the immune suppressive PCV2. PPV4, although not present in every herd tested, showed wide territorial distribution, without obvious epidemiological link among the infected farms. The number of PPV3 cases is increasing according to the study. There was a sudden multiplication of positive cases during 2007 and 2008 and by 2009 the incidence of the infection was around 40 % like in Asia where it was first identified. The genomes of the Hungarian HoKo viruses were not fully identical to those described elsewhere, showing that the presence of the virus was not due to a recent spread from infected areas.

Acknowledgements

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DETECTION AND SEQUENCING OF PORCINE PARVOVIRUS 4 IN SWINE IN GREAT BRITAIN

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Introduction

Recently a new parvovirus of swine, porcine parvovirus 4 (PPV4) has been reported in the USA and subsequently in China. The viral genome was originally detected in samples from a herd in North Carolina with an acute onset disease of high mortality, specifically from the lung lavage of a diseased pig co-infected with PCV2 (1). In China PPV4 was detected by PCR in 2.09% (n=573) of diseased and 0.76% of health pigs tested (n=133) (2). The PPV4 genome is unusual in that the organisation consists of three genes including a small central ORF, similar to the bocaviruses, yet the genetic homology to the bocaviruses is minimal. Instead the virus known to share the closest nucleic acid homology with PPV4 is bovine parvovirus 2 (BVP2), a member of the genus

Materials and methods

44 sera samples were collected from pigs on PMWS-affected UK farms. Tissues were taken from 63 PMWS-affected pigs and 55 non PMWS-affected pigs from the UK and Republic of Ireland. Tissues included lung, liver, kidney, spleen and lymph nodes. Viral nucleic acids were extracted from sera and 10% homogenised tissue samples using Roche MagNA Pure LC. All tissue and sera samples were analysed by real-time SYBR Green I PCR for the presence of PPV4 genome using primers designed using the original PPV4 sequences (GQ387500, GQ387499). Positive samples were confirmed by sequencing. Prevalence of PPV4 was related to post-weaning multisystemic wasting syndrome (PMWS) status where possible. One sample was selected for sequencing of the capsid gene. Sequencing was carried out using standard methods followed by analysis with Vector NTI software. Phylogenetic analysis was carried out using ClustalW 1.81 and Mega4 (3).

Results

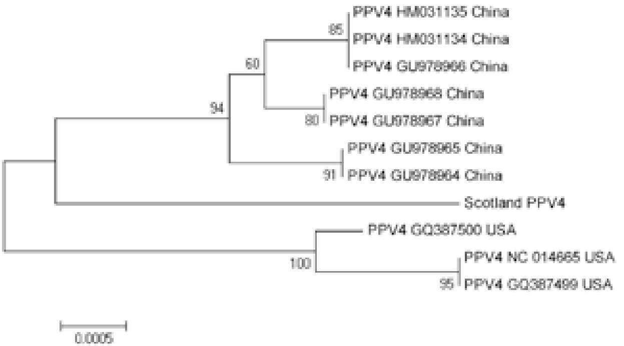
PPV4 was detected in the tissues of six animals in England, from four farms. The virus was detected in the sera of four animals from one farm in Scotland and two animals from two farms in England. No PPV4 was detected in any sample from animals in Northern Ireland or the Republic of Ireland. These results are shown in Table 1. The virus was twice as prevalent in tissues from PMWS affected animals. As estimated from Ct values the virus was present in widely variable amounts.

Almost the entire capsid gene was sequenced (2166 nucleotides). ClustalW 1.81 analysis showed that the sample chosen for sequencing (sera, Scotland) was 99% similar to the US and China sequences. A Mega 4, bootstrapped neighbour joining tree is shown in Fig 1.

Table 1. Prevalence of PPV4 in porcine tissue and sera

Sample	PMWS status	PPV4
Tissue	PMWS +ve	6.3% (4/63)
	PMWS –ve	3.6% (2/55)
	Unknown	0.0% (0/34)
	Total	3.9% (6/152)
Sera	PMWS farms	13.6% (6/44)

Fig 1. Phylogenetic comparison of UK PPV4 with sequences from USA and China.



Discussion

PPV4 was detected in a range of tissues and in sera from pigs in England and Scotland. Phylogenetic analysis showed high levels of homology between the Scottish virus and those from the US and China. There was a significantly higher prevalence in the tissues of PMWS +ve pigs than PMWS –ve pigs, but the overall numbers of PPV4 +ve samples was low. To date the significance of PPV4 in terms of porcine disease is unknown.

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DETECTION AND SEQUENCE ANALYSIS OF A PORCINE BOCAVIRUS (SWPBOV) IN COMBINATION WITH PCV2 AND TTV IN PMWS-AFFECTED AND NON-AFFECTED PIGS IN THE UK

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Introduction

Recent advances in sequencing methods have lead to the discovery of a boca-like virus in Swedish pigs suffering from postweaning multisystemic wasting syndrome (1). As expected this virus was discovered in combination with porcine circovirus type 2 (PCV2). Torque teno virus (TTV) was also present albeit to a lesser extent. Although PCV2 is acknowledged as the causative agent of PMWS (2) it is known that other agents contribute to onset and severity of disease. Subsequent research has associated this porcine bocavirus (SwPBoV) with respiratory tract infections in weanling piglets in China (3) and was demonstrated to have an increased incidence in combination with TTV in PMWS-affected pigs in Sweden (4). It might therefore be possible that this newly discovered SwPBoV acts as a co-factor in the development of PMWS.

In this study the distribution of PCV2, TTV and SwPBoV in archival tissue samples recovered from pigs from 3 UK farms in 2002 was investigated. Due to the recent publication of full coding sequence for SwPBoV (5) we also investigate the sequence similarity between Chinese, Swedish and UK isolates.

Materials and methods

21 pigs from 3 PMWS-affected farms had tissue samples collected. Tissues included lung, liver, kidney, spleen and lymph nodes from each pig. Viral nucleic acids were extracted from homogenised tissue samples. All tissue samples were analysed by PCR for the presence of PCV2 using ORF2 specific primers (2). Samples were also assessed for the presence of genogroup 1 & 2 TTV (TTV1 & TTV2) (6) and SwPBoV (1) using real-time PCR. TTV1 was detected using a set of primers kindly provided by Dr. Annette Mankertz (Robert Koch-Institut, Berlin, Germany) (data not published). The NP1 gene of SwPBoV positive tissues derived from 9 pigs was sequenced and compared to SwPBoV sequences available from GenBank.

Results

Percentage distribution of viruses detected in the samples are detailed in Table 1.

50% of PMWS-affected pigs had all viruses present. 100% of these pigs had PCV2, SwPBoV and either TTV1 or TTV2. Only 27% of non-PMWS-affected pigs had all viruses present simultaneously and 64% of these pigs had PCV2, SwPBoV and either TTV1 or TTV2. Comparison of the UK isolate's NP1 region similarity using ClustalW 1.81 demonstrated that they were ≥97% similar at nucleotide level to the NP1 region of the newly submitted Chinese sequence and to the NP1 region of 6 Swedish isolates.

Table 1. Distribution of PCV2, TTV & SwPBoV in PMWS-affected & non-PMWS-affected tissues from PMWS- affected farms (%).

	PCV2 +VE	TTV1 +VE	TTV2 +VE	SwPBoV +VE
PMWS +VE	100%	60%	90%	100%
PMWS -VE	100%	64%	46%	82%

Discussion

A similar study demonstrated that 71% of affected pigs were simultaneously infected with TTV1, TTV2 and SwPBoV, whereas 33% of non-affected pigs were infected with the 3 viruses (4). This study suggests a similar trend whereby PCV2, SwPBoV and either TTV1 or 2 are more prevalent in PMWS-affected pigs. Further analysis will be required to assess if incidence of a combination of these viruses can be significantly related to disease status. Analysis of the similarity of the NP1 gene of SwPBoV also demonstrated that there is limited diversity between isolates from geographical separated populations. Further analysis of isolates from a wider geographical distribution is ongoing to determine PCV2 and TTV incidence in combination with SwPBoV and to fully investigate SwPBoV sequence diversity.

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PHYLOGENETIC ANALYSIS OF TWO NOVEL PORCINE BOCAVIRUSES (PBOV3 & PBOV4) ISOLATED IN CELL CULTURE FROM SWINE IN NORTHERN IRELAND

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Introduction

Recent research carried out in Northern Ireland has resulted in the isolation in cell culture of 2 novel porcine bocaviruses (PBoV3 and PBoV4). Bocavirus-like virus species have also been discovered in pigs from Sweden and in China (1,2). Characterisation of Chinese isolates has resulted in full length coding sequence for 2 viruses designated as Porcine Bocavirus 1 and 2 (PBoV1 (HM053693) and PBoV2 (HM053694)) (2). This Chinese study also produced partial NP1/VP1 sequence for 2 other bocaviruses designated as 6V Chn (HM053672) and 7V Chn (HM053673). A separate Chinese study detected and fully sequenced another bocavirus originally identified in post weaning multisystemic wasting syndrome (PMWS) -affected pigs from Sweden (1,3). This porcine bocavirus will be referred to here as SwPBoV (HQ223038). In this study we detail the phylogeny of recently identified porcine bocaviruses including those recovered from Northern Irish farms.

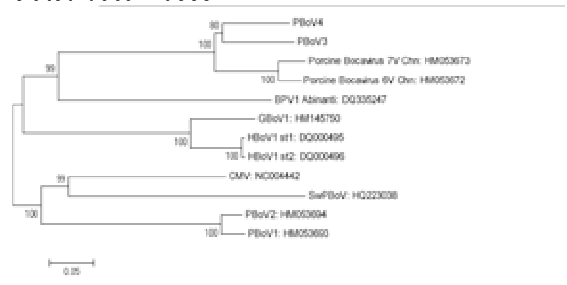
Materials and methods

Both viruses were recovered from material collected from PMWS-affected farms. PBoV3 was identified in a 10% homogenate of small intestine, and PBoV4 was isolated from a faecal swab. Both were passaged in primary pig kidney cells. Cell culture demonstrating cytopathic effect (CPE) was screened by real-time PCR for the presence of PCV1, PCV2, PPV, porcine enterovirus types 1, 2 and 3, porcine adenovirus and porcine reovirus. Virus pools of PBoV3 and 4 were purified using sucrose and CsCl gradient purification. Nucleic acids were extracted from the virus pools using phenol/chloroform extraction. Extracted material was fragmented using restriction enzymes and cloned into pUC19. Genome fragments were sequenced and aligned. Phylogenetic comparison was made with current porcine bocaviruses and bocaviruses from other species including BPV-1, CMV, human bocavirus (HBoV) and gorilla bocavirus (GBoV1) using Mega 5 genetic analysis software. A bootstrapped neighbour joining tree is shown in Fig 1. Virus homology was compared using ClustalW 1.81.

Results

Cloning and sequence analysis resulted in 5228bp sequence data for PBoV3 and 5144bp for PBoV4 which included 3 putative open reading frames. Phylogenetic analysis is detailed in Figure 1. Clustal analysis has indicated that at nucleotide level PBoV 3 and 4 are 80% similar. PBoV 3 and 4 are between 41-51% similar to those bocaviruses analysed including PBoV 1 and 2 but excluding porcine bocavirus NP1/VP1 sequence fragments 6V Chn (HM053672) and 7V Chn (HM053673) (2). In this case PBoV 3 and 4 demonstrated 76-80% homology with these partial sequences recovered from Chinese pigs.

Fig 1. Phylogentic comparison of PBoV 3 and 4 with porcine and related bocaviruses.



Discussion

The 2 bocavirus isolates described in this study represent 2 distinct bocavirus species. We are the first laboratory to report that swine bocaviruses have been adapted to grow in tissue culture. Phylogenetic analysis demonstrated that PBoV 3 and 4 share relatively high homology with Chinese partial sequence fragments 6V and 7V. However, the 2 novel bocaviruses described here are approximately 40-50% similar to other porcine bocaviruses. The pathogenicity of PBoV 3 and 4 has not yet been determined but, related swine bocaviruses have been linked to respiratory illness (4) and PBoV3 was isolated from a piglet suffering from diarrhoea and pneumonia. PBoV3 and 4 might not be directly attributable to disease but might function as co-factors in the development of other porcine diseases. Research is currently ongoing to determine the prevalence and pathogenic potential of these novel bocaviruses.

Acknowledgements

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PRODUCTION, CHARACTERISATION AND APPLICATIONS OF MONOCLONAL ANTIBODIES TO TWO NOVEL PORCINE BOCAVIRUSES (PBOV3 & PBOV4) ISOLATED IN CELL CULTURE FROM SWINE IN NORTHERN IRELAND

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Introduction

Bocavirus-like virus species have been discovered in post weaning multisystemic wasting syndrome affected pigs in Sweden (SwPBoV) (1) and healthy piglets in China (PBoV1 and PBoV2) (2,3). Field studies in Northern Ireland have resulted in the isolation of 2 novel porcine bocaviruses (PBoV3 and PBoV4) from PMWS-affected farms. This study describes the production of monoclonal antibodies (mAbs) to PBoV3 and PBoV4, their preliminary characterisation and possible applications.

Materials and methods

Both viruses were isolated in primary pig kidney cells and repeatedly passaged to give an evident cytopathic effect (cpe). Material was then purified using standard methods to the level of sucrose gradient differential centrifugation and used as inoculum for BALB/c mice. After 3-4 weeks mice were boosted with similar inoculum and spleens harvested after 4 days. Mouse spleen cells were fused with non-secretory mouse myeloma cells using modified standard procedures (4). Screening was carried out by indirect immunofluorescence (IIF) on multispot slides cultured with PBoV3 and PBoV4 infected cells. Positive colonies were cloned twice by limiting dilution and final clones expanded into flasks. Supernatant was harvested and stored as mAbs. Two mAbs from each fusion were selected for further investigation. A subsequent fusion was carried out using PBoV3 as initial inoculum followed by a boost with PBoV4. Screening of emerging colonies was carried out against both viruses. No further work was carried out on these colonies.

Immunoglobulin class of each mAb was determined by IIF using sub-class specific FITC-labelled secondary conjugates on acetone fixed coverslips of homologous virus.

Cross-reactivity of each mAb against homologous and heterologous virus was measured by IIF. mAbs were titrated and titre compared against PBoV3 and PBoV4 infected cells.

Evidence of neutralising activity was tested by inoculation of 10 fold dilutions of virus previously incubated with homologous mAb into 24 well plates containing primary pig kidney cells and sterile glass coverslips. After 48 hours coverslips were harvested, fixed and stained by IIF. The titres were compared to 10 fold virus dilutions incubated without mAb.

One mAb from each fusion was selected to use in the development of 2 antigen-capture ELISA's specific for PBoV3 and PBoV4. In brief plates were coated with a PBoV3 or PBoV4 polyclonal rabbit serum prior to addition of virus sample. Bound virus was detected by the mAb and visualised by means of a biotin-extravidin-peroxidase technique

Results

All mAbs were of the IgG2a isotype.

There was no evidence of cross-reactivity between PBoV3 and PBoV4 mAbs/virus.

Partial neutralisation was observed between PBoV4 mAbs and virus with a fall in titre of 1.5 logs. There was no reduction with PBoV3 mAbs and virus.

The specific antigen-capture ELISA's were successful at detecting peak virus fractions in sucrose gradients. This was confirmed by electron microscopy and virus replication in primary pig kidney.

Discussion

The absence of cross-reactivity between the mAbs/virus would suggest 2 very distinct strains and this is confirmed by efforts to produce a "pan-boca" mAb using a combination of both isolates. Although secreting colonies were detected none were positive for both viruses.

Although the pathogenicity of swine bocaviruses is largely undetermined SwPBoV has been linked to respiratory illness and PBoV3 was isolated from a piglet suffering from diarrhoea and pneumonia. Further investigation is required to determine if swine bocaviruses identified to date can cause disease in their own right or are present as contributors in multifactorial diseases.

To date there have been no other reports in literature of the isolation and cultivation of swine bocavirus and subsequent production of reagents. Such reagents would be useful in the study of the biology of these viruses, the diagnosis of infection and investigations into the pathogenesis of potential swine bocavirus associated diseases.

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DETECTION OF TWO NOVEL BOCAVIRUS SPECIES (PBOV3 & PBOV4) IN SWINE USING REAL-TIME PCR

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Introduction

Two distinct porcine bocaviruses (PBoV3 and PBoV4) have been isolated from pigs in Northern Ireland. The viruses were isolated in primary cell culture, from the small intestinal tissue and a faecal specimen from two pigs on different PMWS-affected farms. Although the clinico-pathological significance of PBoV has yet to be determined, the discovery of swine boca-like virus warrants further investigation given that bocavirus infection is already associated with a number of disorders in other species (1).

Recently, the discovery of a swine boca-like virus (SwPBoV) in Swedish pigs suffering from postweaning multisystemic wasting syndrome has been reported (2). SwPBoV has subsequently been detected in weaning piglets with respiratory tract infections in China (3). Here we describe the development and application of a real-time PCR assay for the detection of PBoV3 and PBoV4 DNA.

Materials and methods

Primers were designed against conserved nucleotide sequences of PBoV3 and PBoV4. Real-time PCR was performed on an MJ Research Opticon 2 instrument using SybrGreen PCR master mix (Qiagen, West Sussex, UK) in 25µl reaction volumes containing 2µl of template. Assay optimisation involved the determination of optimum Ta, Mg^{2+} and primer concentrations. Assay sensitivity was assessed against serial dilutions of quantified amplicons of PBoV3 and PBoV4 nucleic acid while assay specificity was evaluated against a number of cell lines, porcine and non-porcine viruses.

The assay was applied to tissue samples of pigs submitted to the post-mortem department, AFBINI, Stormont, Northern Ireland. 10% (w/v) tissue homogenates were prepared using standard methods and nucleic acid was extracted from homogenates (200µl) using the MagNA Pure LC Total nucleic acid extraction kit (Roche, Burgess Hill, UK). Representative amplicons (2.8Kb), derived from both PBoV isolates served as positive control DNA.

Results

PCR amplification was achieved using 0.6µM primer concentration and 40 cycles of 95°C for 30 sec, 52°C for 30 sec, 72°C for 30 sec followed by a melt-curve between 60 – 95°C. PCR products of the predicted size (313-316bp), with Tm of approximately 79°C and 78°C were determined for PBoV3 and PBoV4, respectively. The specificity of the assay was confirmed against a range of cell lines and DNA viruses. The assay was applied to a number of field samples which included lung, spleen, mesenteric lymph node and tonsil. Fig. 1 shows a selection of PBoV positive samples, with Tm ranging from 78.7 to 79.5.

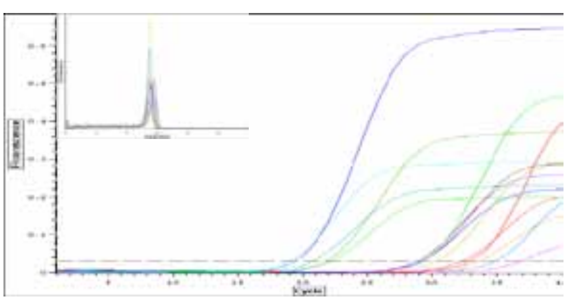

Fig. 1 Fluorescence v cycle no. for PCR assay run against porcine tissue samples. Melt curve is shown in inset.

PBoV DNA was most frequently detected in the mesenteric lymph nodes and was also present in a proportion of lung, spleen and tonsillar tissue. In some cases, PBoV was detected in 2-3 different tissues of each animal from the same farm. These results, coupled with the fact that the animals included in this study were from different geographical locations, would suggest that PBoV is circulating within the swine population in Northern Ireland.

Discussion

To date, the detection of PBoV DNA has not been directly associated with clinico-pathological effects in swine. However, the development of a PBoV PCR assay provides a means by which viral prevalence, epidemiology and the genetic diversity of PBoV within swine populations can be further investigated. Virus isolation in primary cell culture will be performed on the PBoV-positive samples for the purposes of conducting future serological studies and/ or experimental infections. Sequencing analyses will also be conducted to establish the genetic relationship between the PBoV detected in this study and other known swine bocavirus sequences.

Acknowledgements

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DETECTION AND SEQUENCING OF PORCINE HOKOVIRUS IN SWINE IN THE UK AND REPUBLIC OF IRELAND

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Introduction

A novel parvovirus of swine, porcine hokovirus (PHoV) has been reported in Hong Kong (1) and subsequently in Germany (2). PHoV has been provisionally assigned as a member of the putative genus Hokovirus within the family Parvoviridae along with bovine hokovirus (BHoV) and human parvovirus 4 (PARV4). PHoV is a non-enveloped single stranded DNA virus, approximately 5kb in length. The genome has two open reading frames coding for the capsid and non-structural proteins. The viral genome was originally detected in porcine samples (including lymph nodes, liver, serum, nasopharyngeal and faecal) from Hong Kong; PHoV was detected by PCR in 44.4% (n=333) of pigs tested (1). In Germany (2), PHoV was identified in the wild boar populations, again using pcr, and analysis showed a prevalence of 32.7% (n=156) in liver and sera samples with the prevalence differing between German regions and with increasing age of the animal.

Materials and methods

Tissues were taken from 63 PMWS-affected pigs, from 55 non PMWS-affected pigs, and from 34 pigs of unknown PMWS status from the UK and Republic of Ireland (RoI). Tissues included lung, liver, kidney, spleen and lymph nodes. 44 sera samples were collected from pigs on PMWS-affected UK farms. Viral nucleic acids were extracted from 10% homogenised tissue samples and sera using Roche MagNA Pure LC. All tissue and sera samples were analysed by real-time SYBR Green I PCR for the presence of PPV genome using primers designed based on the original PHoV sequences (EU200667-EU200677). Prevalence of PHoV was related to post-weaning multisystemic wasting syndrome (PMWS) status where possible. One sample was selected for sequencing of the capsid gene which was carried out using standard methods followed by analysis with Vector NTI software. Phylogenetic analysis was carried out using ClustalW 1.81 and Mega4 (3).

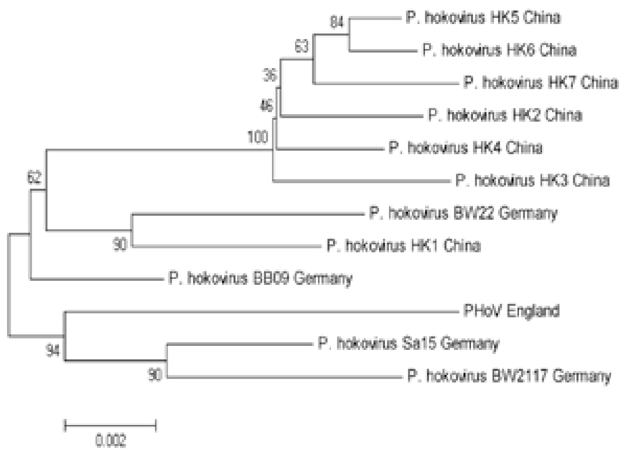
Results

PHoV was detected in the tissues of nine animals in the UK/RoI from seven farms. The virus was detected in the sera of three animals from one farm in Scotland and four animals from one farm in England. These are shown in Table 1. As estimated from Ct values the virus was present in widely variable amounts. The partial capsid gene was sequenced (2372 nucleotides). ClustalW 1.81 analysis showed that the sample chosen for sequencing (sera, England) was 94-98% similar to the Hong Kong and German sequences. A Mega 4, bootstrapped neighbour joining tree is shown in Fig 1.

Table 1. Prevalence of PHoV in porcine tissue and sera

Sample	PMWS status	PHoV
Tissue	PMWS +ve	3.2% (2/63)
	PMWS –ve	9.1% (5/55)
	Unknown	5.9% (2/34)
	Total	5.9% (9/152)
Sera	PMWS +ve farms	15.9% (7/44)

Fig 1. Phylogenetic comparison of UK PHoV with sequences from Hong Kong and Germany.



Discussion

PHoV was detected in a range of tissues and in sera from pigs in the UK and the Republic of Ireland (RoI). Phylogenetic analysis showed high levels of homology between the UK/RoI virus and those from Hong Kong and Germany. There was a higher prevalence in the tissues of PMWS -ve pigs than PMWS +ve pigs, but the overall numbers of PHoV +ve samples was low. To date the significance of PHoV in terms of porcine disease is unknown.

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EMERGENCE OF PSEUDORABIES VIRUS INFECTION IN HUNTING DOGS AND FIRST REPORT OF DIRECT TRANSMISSION FROM THE WILD BOAR POPULATION IN AUSTRIA

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Introduction

Pseudorabies virus (PrV, SuHV-1) is the causative agent of Aujeszky´s disease (AD), an OIE notifiable disease of domestic pigs with high economical importance. Only members of the *Suidae* family can survive the infection as virus reservoirs, while other mammalian species, except primates and humans, succumb to progressive and fatal encephalitis (1, 2, 3). In recent years, PrV clinical infection of hunting dogs has been repeatedly diagnosed in Austria: one case in 2004 (2), two in 2008 and 4 cases since November 2010 (3). AD in hunting dogs was recently also reported by other countries (4), and seroprevalence studies in the European wild boar population (5, 6, 7) have shown that the disease is more widely distributed than previously assumed (4). Infection of wild boars in Lower Austria (LA) and epidemiologically related AD in hunting dogs was demonstrated at the molecular and serological level. As a novelty, PrV infection in both, the dead hunting dog and the ingested tissues from the wild boar, was investigated.

Materials and methods

Following necropsy, brain tissues of 4 hunting dogs, suspected to have died from AD, were sampled for histology and immunohistochemistry, virus isolation and molecular detection by PrV real-time PCR. All dogs had a history of feeding on freshly shot wild boars during hunting. From 71 wild boars shot during hunting season in Lower Austria, lung, lymph nodes, spleen and tonsil samples were analysed by PrV real-time PCR. PrV DNA detection in pooled samples was compared to DNA extracted from tonsils alone. Real-time PCR positive samples were confirmed by gB nested PCR and sequencing.

Results

Histology from the dog brain samples revealed lymphohistiocytic encephalitis with neuronal necroses and demonstration of intranuclear inclusion bodies. In the PrV-positive wild boars, aside from tonsillar necroses, no further lesions were detected.

PrV DNA was detected by real-time PCR in the brain of all dead dogs at Cq values ranging from 23.0 - 34.9. From 71 obviously healthy wild boars, two animals were positive. Tissue pools and tonsils of these two animals gave mean Cq values of 35.32 and 28.9, respectively. In one animal, only the tonsil sample was positive, indicating that pooling may decrease the sensitivity of detection in asymptomatic animals and that tonsils should always be sampled for PrV detection in wild boar. This was confirmed by the fact that the tonsil sample of one of the wild boars, contained a higher viral load than the tongue base or the nasal mucosa (lymph nodes, kidney, muscle and salivary gland were negative). Sequence analysis of the gB gene indicated the presence of at least two different PrV field strains. From one dog, PrV was isolated on the PK-15 cell-line and confirmed by immunofluorescence.

Discussion

Since 1997, Austria is officially recognized free from AD in domestic swine, and all serological investigations performed on domestic swine samples in the framework of a national screening program have been negative. However, our findings demonstrate the presence of PrV infection in the wild boar population. Although direct transmission from wild boars to carnivores after consumption of infected carcasses has been presumed (4), we present direct evidence of PrV infection and consecutive AD in a hunting dog following ingestion of wild boar tissue. Further molecular epidemiological investigations of the PrV strains involved may shed light on the origin of the infection. Moreover, future surveillance programs are planned to assess the distribution of PrV in the Austrian wild boar population.

Acknowledgements

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A NOVEL ROLLING CIRCLE AMPLIFICATION ASSAY TO DETECT MEMBERS OF THE FAMILY ANELLOVIRIDAE FROM PIG AND HUMAN

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Introduction

Members of the family Anelloviridae are vertebrate-infecting, single-stranded circular DNA viruses divided in 9 genera based on their host species (1). In swine two genetically distinct genogroups are described, namely Torque teno sus virus 1 (TTSuV1) and 2 (TTSuV2) (2). It is probable that novel swine genogroups exist as shown in the human TTV counterpart.

Rolling-circle amplification (RCA) is a novel technique used to amplify circular DNA templates using random hexamers, yielding 10^9 or more copies of a circular sequence (3).

The aim of the present study was to optimize a novel RCA method using Anellovirus-specific primers (Anello-RCA) to detect members of the genus Iotatorquevirus in swine and Alphatorquevirus in human biological samples, and apply it to sera from other animal species.

Materials and methods

A TTSuV2 positive swine serum sample by conventional PCR (4) and 5 human sera TTV positive by real-time quantitative PCR (5) were selected to optimize the Anello-RCA technique. After technical optimization, 10 swine and 5 human sera PCR negative for TTVs were tested. Furthermore, 123 serum samples from animal species including chicken (49), bovine (68) and sheep (6), whose TTV sequences have not been fully characterized yet, were tested.

Anellovirus-specific primers were designed corresponding to a highly conserved region among all full-length Anelloviridae genomes from several species available in the GenBank. Anello-RCA was optimized using short Anello-specific primers (6 nt) and the resulting dsDNA products, that are tandem repeat copies of the viral complete genome, were used as templates in inverse-PCR with Anello-specific PCR primers (12 and 15 nt in length). PCR products purified from agarose gel were sequenced.

Results

Anello-RCA/PCR assay resulted in fragments between 2 and 3 kb of length in swine and between 3 and 4 kb of length in human. From 10 PCR negative swine sera, 3 TTSuVs (one TTSuV1 and two TTSuV2) could be identified. The shorter not expected fragments from swine sera observed on agarose gel were bacterial genome and host DNA sequences. In addition, 2/5 human sera were TTV positive with the highest similarity to TTV isolate tth14 (97% similarity) and TTVyon-LC011 (90% similarity). No amplication was achieved in any of the samples corresponding to chicken, bovine and sheep.

Discussion

The present study demonstrates that the optimized Anello-RCA/PCR technique is a reproducible tool for anellovirus detection in sera from different species. The advantages of the technique are: a) the handling is very simple; b) no expensive devices are necessary; c) the costs per reaction are very low; and d) multiple copies of viral complete genome could be easily obtained. Due to the short nature of the Anello-primers, other templates were also amplified, suggesting that the Anello-RCA/PCR would not be so strictly specific to *Anelloviridae* genomes. However, the amplicons corresponding to other templates were always shorter than expected TTV full-length genomes and easy to discriminate from subsequent analyses.

Attemps to amplify anelloviruses from chicken, bovine and sheep serum were not successful. As in amplifying TTSuV and TTV genomes full-length Anello-PCRs were properly optimized, further different reaction conditions for detecting anelloviruses in the other species should be tested.

Acknowledgements

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THE FIRST REPORT OF PRESENCE OF TORQUE TENO VIRUS (TTV) IN PIGS IN CROATIA

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Introduction

Torque teno virus (TTV) is non-enveloped, contains a circular, single-stranded DNA genome and belongs to the family *Anelloviridae*. [1] More recently, TTV was also identified in many domestic animals, including swine [1]. Two different TTV species, members of *Iotatorquevirus* genus have been described so far in domestic pigs and wild boars, *Torque teno sus virus* 1 (TTV1) and *Torque teno sus virus* 2 (TTV2) [1, 2]. Swine TTV is widespread virus [3] and until now it was considered as non-pathogen but it was confirmed as a trigger to PCV2 in pathogenesis for porcine multisystemic wasting syndrome (PMWS) [4]. The same group of authors make connection between infection in swine and porcine dermatitis nephropathy syndrome (PDNS) [5]. The aim of this study was to detect presence of TTV in pigs in Croatia and possible connection to the PCV2 associated diseases.

Materials and methods

Altogether 85 samples of isolates of DNA (extracted by QIAamp®DNA Mini Kit, Qiagen, Hilden, Germany) were submitted to the PCR for detection of presence of TTV1 and TTV2 according to the protocol as previously described [6]. All samples were PCV2 positive and coming from:

63 of samples fetuses from reproductive disorder (RD) cases, 14 samples of lymph nodes from pigs affected with interstitial nephritis and nephrosis (IN), 3 samples of lymph nodes from wild boar PMWS and 5 samples of lungs from proliferative and necrotizing pneumonia (PNP).

Results

According to the 85 collected samples, in this study we found 7 (8,23%) samples positive for presence of TTV1 and 17 (20%) samples positive for presence of TTV2.

Origin of samples	TTV1 positive	TTV2 positive
RD (No. 63)	1 (1,58%)	2 (3,17%)
IN (No. 14)	1 (7,14%)	8 (57,14%)
Wild boar PMWS (No. 3)	3 (100%)	3 (100%)
PNP (No. 5)	1 (20%)	4 (80%)

Discussion

In this study we confirmed presence of both genotypes of TTV in domestic pigs and wild boars. It is hard to believe that TTV is involved in pathogenesis of reproductive disorders because we detected a low prevalence. However there is strong indication that TTV2 can be involved in pathogenesis of PNP and IN. Both genotypes of TTV were detected in wild boar PMWS cases. It is known that TTV can be trigger for PMWS in domestic pigs and it isn't unexpected to be a trigger in wild boars PMWS also. Future study must estimate real prevalence TTV in Croatia and investigate involvement of TTV in pathogenesis of different syndromes like PNP and IN.

Acknowledgements

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TORQUE TENO SUS VIRUS 1 AND 2 PREVALENCE AND VIRAL LOADS IN PIGS EXPERIMENTALLY INFECTED WITH CLASSICAL SWINE FEVER VIRUS

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Introduction

Torque teno viruses (TTVs) are small, non-enveloped viruses with a circular single-stranded DNA genome, belonging to the family *Anelloviridae*. In swine, two species (Torque teno sus virus [TTSuV] 1 and 2) have been identified and grouped within the genus Iotatorquevirus (1).

Pathogenic role of TTSuVs is still under debate. To date, different studies have linked TTSuV2 to postweaning multisystemic wasting syndrome (PMWS) affected pigs (2,3), and TTSuV1 to PMWS and a porcine dermatitis and nephropathy syndrome (PDNS)-like condition (4,5). These associations point out a pathogenic role of TTSuVs or may indicate that TTSuVs replication is up-regulated under disease conditions. In order to further explore the association of TTSuVs with disease occurrence, the aim of this work was to evaluate TTSuVs prevalence and viral load before and after an experimental infection with a highly pathogenic classical swine fever (CSF) virus.

Materials and methods

Sera from 41 animals corresponding to two CSF virus experimental studies (6) were used. Animals were grouped into 3 categories based on their immune response to CSFV infection and CSF clinical outcome, Group I (n=23) included immunized pigs showing moderate to severe CSF signs. Group II (n=6) was composed by immunized animals displaying mild CSF symptoms. Finally, group III (n=12) included non-immunized animals that suffered from severe signs of CSF disease, with early death.

All sera were tested by quantitative PCR (qPCR) for TTSuVs (3) at two time points: before CSF virus challenge (T0) and at necropsy (TN) (between 7 and 13 days after challenge).

TTSuV1 and TTSuV2 prevalence and viral loads (log 10 copies/ml of serum) were compared before and after CSF virus challenge with contingency tables and ANOVA, respectively. Statistical significance level was set at $p<0.05$.

Results

Results of qPCR and prevalence for TTSuV1 and 2 in the different animal groups are summarized in Table 1. TTSuV2 load increased significantly globally (and specifically in Group I pigs) from T0 to TN. No significant differences were observed globally or in any group for TTSuV1.

Table 1. TTSuV1 and TTSuV2 mean viral loads (log10 copies/ml of serum) and number of infected pigs (in parentheses) in the different studied groups, before CSF virus challenge and at time of necropsy.

	TTSuV1		TTSuV2	
	T0	TN	T0	TN
Group I (23)	5,061 (14)	4,903 (12)	5,395[a] (20)	5,875[b] (23)
Group II (6)	5,074 (6)	5,177 (6)	5,185 (6)	5,329 (6)
Group III (12)	4,215 (5)	4,302 (2)	5,017 (10)	5,206 (10)
Total (41)	4.895 (25)	4.925 (20)	5.255[a] (36)	5.619[b] (39)

[a,b] Different superscripts mean significant differences for mean viral load comparing T0 and TN.

Discussion

CSF is a highly contagious viral disease causing immunesuppression and high mortality rates (6). Taking into account that up-regulation of TTSuVs in porcine circovirus diseases might be linked to an immunosuppressive status, this study was aimed to explore whether a disease that strongly affects the immune system would be able to affect prevalence and loads of TTSuVs in serum of pigs naturally infected with these viruses. Results indicated that TTSuV2 load in serum increased after challenge with CSF virus, mainly in the group of pigs with moderate to severe clinical signs. Therefore, this study also emphasizes the different behaviour of both TTSuVs and further supports the association of TTSuV2 with disease occurrence.

Acknowledgements

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TORQUE TENO SUS VIRUS TYPE 1 (TTSUV1) AND 2 (TTSUV2) PREVALENCE AND VIRAL LOADS IN SERUM OF POOR-DOING ANIMALS

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Introduction

Torque teno viruses (TTVs) are small, non-enveloped viruses with a circular single-stranded DNA genome, belonging to the family *Anelloviridae*. In swine, two species (*Torque teno sus virus* [TTSuV] *1* and *2*) have been identified and grouped within the genus *Iotatorquevirus* (1). No clear-cut pathogenic role of TTSuVs has been demonstrated so far, but their potential pathogenicity is under debate. In fact, it has been shown that TTSuV2 prevalence and viral loads are higher in postweaning multisystemic wasting syndrome (PMWS) affected pigs (2,3). In addition, TTSuV1 has been linked to PMWS and a porcine dermatitis and nephropathy syndrome (PDNS)-like condition (4,5). Increased prevalence and loads of TTSuVs in diseased animals may be directly related to their pathogenic role or, alternatively, their replication be up-regulated by immune dysfunction. Therefore, the aim of the present study was to compare TTSuV1 and TTSuV2 viral loads and prevalence in serum of healthy and wasted pigs of unknown aetiology.

Materials and methods

Twelve wasted pigs with growth-retardation and low body mass index (mean weigh = 6.13 kg) and 12 age-matched, healthy pigs (mean weigh = 15.3 kg) were selected at a nursery phase from a farrow-to-finish farm. Sera of these animals were tested for both TTSuV DNA loads by quantitative PCR (qPCR) and haptoglobin (Hp) levels by ELISA. TTSuV1 and TTSuV2 prevalence and viral loads were compared with contingency tables and ANOVA respectively, with a statistical significance level at $p<0.05$. A correlation test was applied to individual viral loads and Hp levels.

Results

TTSuV1 and TTSuV2 prevalence in both studied pig groups are displayed in Table 1. TTSuV2 was significantly more prevalent in poor-doing animals than healthy pigs. No significant differences were found in TTSuVs mean viral loads in healthy and wasted piglets.

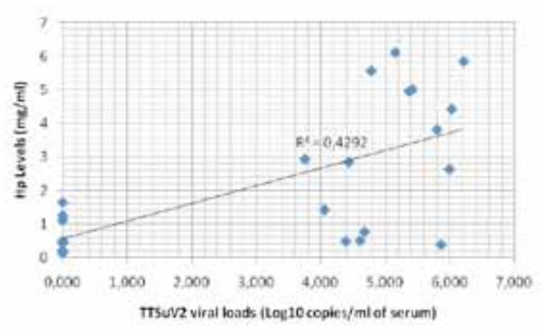
Hp mean level was statistically higher in wasted animals (3.61 mg/ml) than in healthy ones (0.85 mg/ml). A strong positive correlation was found when comparing TTSuV2 individual viral loads and Hp levels. This relation was not found for TTSuV1.

Table 1. TTSuV1 and TTSuV2 mean viral loads (log10 copies/ml of serum) and prevalence (percentage) in healthy and wasted animals.

	TTSuV1		TTSuV2	
	Healthy	Wasted	Healthy	Wasted
Viral load	3,896	4,128	4,725	5,286
Prevalence	75	83	42[a]	83[b]

[a,b]Different superscripts mean significant differences.

Figure 1. Correlation of Hp levels and TTSuV2 viral loads.



Discussion

Results from this study showed that TTSuVs were highly prevalent among studied piglets. Even though we found no differences in TTSuV2 viral loads among studied groups, the positive correlation between Hp levels and TTSuV2 viral loads would suggest an association between this virus and an inflammatory systemic state of the animals.

A number of pathogens (porcine reproductive and respiratory syndrome virus, porcine circovirus type 2 or others) could be the main cause of the wasting problem. However, obtained results may suggest a preliminary association of TTSuV2 with background disease status, which deserves further investigation.

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RAPID RATES OF EVOLUTION IN MEMBERS OF THE GENERA IOTATORQUEVIRUS (TORQUE TENO SUS VIRUS 1 AND 2)

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Introduction

Mutation rate is a critical parameter for understanding viral evolution and has important practical implications at both the epidemiological and evolutionary levels. Mutation rate refers to the number of genetic errors that accumulates per unit time, or per generation (1) and is one of the main factors determining the risk of emergent infectious diseases (2).

Various estimates of viral mutation rates have been published, reflecting the changes in nucleotide sequence that occurs during each round of viral replication. These estimates span from $1.5 \cdot 10^{-3}$ mutations per nucleotide, per genomic replication (mut/nt/rep) in single-stranded (ss) RNA, to $1.8 \cdot 10^{-8}$ in the double-stranded (ds) DNA viruses (1, 2).

Torque Teno Virus (TTVs) are circular, single-stranded DNA viruses that infect several vertebrate species (3, 4). In swine, *Torque teno sus virus 1* (TTSuV1) and *2* (TTSuV2) have been so far identified.

The main objective of this work was to obtain a reliable estimation of mutation rate in the two swine infecting viruses within the genus I*otatorquevirus* (TTSuV1 and TTSuV2).

Materials and methods

The mutation rates of TTSuV were estimated using two datasets: a restrospective study including pig sera (n=94) from years 1985 to 2009, and sera from healthy pigs sampled weekly during a 4 week-period.

A genome fragment of 678 bp (TTSuV1) or 719 bp (TTSuV2) of viral genome was amplified, including the untranslated region, the whole ORF2 gene and the 5´end of the ORF1 gene.

Mutation rate estimates were obtained performing three independent runs of Bayesian Markov Chain Monte Carlo (MCMC) chains ($5 \cdot 10^{6}$ generations each) per data set using the software BEAST (Bayesian Evolutionary Analysis Using Trees) (5).

Results

The mutation rate of both TTSuVs was 10^{-3} mut/nt/rep, which is comparable to that of the RNA viruses. All pigs sampled weekly were co-infected with different strains of TTSuVs.

Discussion

The usage of the replicative machinery of the host by the virus has been proposed as an explanation for the high rates of error detected in TTV replication (6). Together, the high rate of mutation and the presence of several strains within a single individual point to the existence of a quasi-species pattern for both species, a fact already observed for other species within the family *Anelloviridae* (7).

However, as has been already indicated for other virus showing similar results (8), additional analyses are needed in order to give consistence to these results.

Acknowledgements

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EXPRESSION PROFILE AND SUBCELLULAR LOCALIZATION OF TORQUE TENO SUS VIRUS PROTEINS

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Introduction

Domestic pig and wild boar infecting *Torque teno sus virus 1* (TTSuV1) and *2* (TTSuV2) are classified into the family *Anelloviridae,* genus *Iotatorquevirus*.

Research on TTSuV has been based almost solely on PCR and only few studies in human TTV have focused on transcription and expression strategy (1,2,3). The present study was aimed to characterize the generation and localization of TTSuV1 and TTSuV2 viral proteins.

Materials and methods

Full-length TTSuV1 and TTSuV2 ORF genes were amplified from TTSuV positive sera and cloned into pcDNA3.1/CT-GFP-TOPO expression vector in frame with GFP. Expression constructs obtained were transfected into porcine kidney (PK)-15 cells.

To characterize possible splicing of studied genes, total RNA was extracted from transfected cells and converted to cDNA. PCRs were performed using viral specific primer pairs.

Subcellular localization of proteins was studied by confocal microscopy. Transfected cells were fixed, labelled against splicing speckle marker (SC-35) and nuclei stained with DAPI

Western blot analysis was performed using total protein extracts from transfected cells and anti-GFP antibody.

Results

TTSuV1 and TTSuV2 ORF1 and ORF3 proteins were accumulated into the nucleus of transfected cells in nucleolar regions (Figure 1). Small globular structures distributed in the nucleoplasm of TTSuV1 ORF1 and TTSuV2 ORF3 transfected cells were observed but did not co-localize with nuclear splicing speckles. ORF2 protein of both viral species was distributed throughout the cell.

Western blot analysis confirmed the presence of expected ORF2 and ORF3 proteins. However, a lower molecular weight ORF1 proteins, compared to the predicted one, was identified.

Transcription profile analysis revealed that ORF1 full length mRNAs and consequently, full length proteins, were not produced. In contrast, 2 and 3 transcripts for TTSuV1 and TTSuV2, respectively, were generated by alternative splicing. ORF2 transcripts obtained for both TTSuV species were of predicted full-length size.

For TTSuV1 ORF3, the expected protein was obtained. However, additional 1 and 2 different transcripts were amplified for TTSuV1 and TTSuV2, respectively.

Subcellular localization of the newly described protein isoforms was also studied and different localizations distribution was determined for TTSuV1 ORF1 and TTSuV2 ORF3 protein isoforms.

Discussion

The present study described, for the first time, the alternative splicing pattern of TTSuV1 and TTSuV2 genes, the protein isoforms produced, and their subcellular localization. Subcellular localization analysis of expressed proteins demonstrated that proteins of genetically distinct TTSuV species localized in the same cellular compartment. Furthermore, similar localization of the human TTV proteins has also been described (1). In spite of the high degree of amino acid divergence (>50%) between human and swine TTVs, viral protein localization is not altered and seems to be maintained in the *Anelloviridae* family.

Acknowledgements

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COMPARISON OF THREE PCR TECHNIQUES FOR DETECTION AND QUANTIFICATION OF TORQUE TENO SUS VIRUSES

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Introduction

Torque teno viruses (TTV) are small, non-enveloped viruses with a circular single-stranded DNA genome. TTVs belong to the family *Anelloviridae* and they are widely distributed in different animal species (1). Two species are known to infect pigs, namely *Torque teno sus virus 1* (TTSuV1) and *2* (TTSuV2), from the genus *Iotatorquevirus*. Currently, TTSuV detection is based on PCR methods.

The objective of this study was to compare the performance of three different PCR assays to detect TTSuVs, being two of them able to quantify them.

Materials and methods

DNA from 33 serum samples corresponding to pigs from different studies was extracted using commercial extraction kits.

The standard PCR used was based on a published technique (2) and was performed by laboratory A. The same laboratory applied a real time quantitative PCR (qPCR) assay based on the Light upon Extension™ (Lux™) technique (3). For quantification, 10-fold serial dilutions (10^8-10^0 genomic equivalents) of cloned full-length TTSuV1 and TTSuV2 genomic clones were used. Laboratory B applied a qPCR assay based on SybrGreen technology (BioRad). Standard curves were based on clones containing partial genomes of TTSuV1 and TTSuV2. Ten-fold standard dilutions were made ranging from 10^7 to 10^1 genomic equivalents. All three techniques were compared in their performance for detection of TTSuV species from serum samples.

Agreement between the different techniques was analyzed by means of Kappa coefficient.

Results

Detection limits for standard PCR were determined to be $5x10^3$ molecules/reaction for TTSuV1 and $5x10^2$ molecules/reaction for TTSuV2. For the D-lux qPCR the sensitivity of the assay was $2x10^3$ copies/ml sera, standard curve accuracy of R^2=0.999, efficiency 99%, intra-assay variation less than 1.3% and inter-assay variation less than 5%. For the SybrGreen qPCR it was calculated an accuracy of R^2>0.999, efficiency for TTSuV1 of 92.5% and 91.4% for TTSuV2, intra-assay variation less than 1.3% and inter-assay variation less than 7%. For the same technique, melting temperature was 84.5ºC for TTSuV1 and 86.7ºC TTSuV2. For both TTSuVs, the detection limit was set to 10 copies/reaction (2.5 x 10^3 copies/ml of sera).

Standard PCR and D-lux qPCR techniques did not cross-react with pig genomic DNA or common pig viruses. Primers used in the TTSuV2 SyberGreen technique cross-reacted with TTSuV1. Nevertheless TTSuV1 primers did not cross-react with TTSuV2.

Results of different methods are summarized in Table 1. Kappa coefficients are included in Table 2.

Table 1. Number of positive sera by the different PCR techniques used

Standard PCR		D-LuX qPCR		SybrGreen qPCR	
TTSuV1	TTSuV2	TTSuV1	TTSuV2	TTSuV1	TTSuV2
22/33 (66.7%)	21/33 (63.6%)	13/33 (39.4%)	18/33 (54.6)%	21/33 (63.6%)	23/33 (69.7%)

Table2. Kappa coefficients (k)* among compared techniques.

	TTSuV1	TTSuV2
Standard PCR vs D-Lux qPCR	0.491	0.689
Standard PCR vs SybrGreen qPCR	0.800	0.457
D-Lux qPCR vs Sybrgreen qPCR	0.542	0.686

*k value =0.41-0.60 (moderate agreement); k value =0.61-0.80 (substantial agreement).

Discussion

Three different techniques were used in a ring-trial to estimate the reliability for TTSuV detection in two different laboratories. Qualitative results (positive or negative results in PCR) were in good accordance between laboratories and techniques. SyberGreen based qPCR showed cross-reactivity of the primers used for TTSuV2 detection. Therefore, obtained quantifications were reliable for the TTSuV1 Sybergreen qPCR and for both species using the D-lux qPCR technology.

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DYNAMICS OF TORQUE TENO SUS VIRUS 1 (TTSUV1) AND 2 (TTSUV2) DNA LOADS IN SERUM OF HEALTHY AND POSTWEANING MULTISYSTEMIC WASTING SYNDROME (PMWS) AFFECTED PIGS

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Introduction

Anelloviruses are small, non enveloped viruses with circular, single stranded DNA genome (1). In swine, two species have been identified so far, *Torque teno sus virus 1* (TTSuV1) and 2 *(*TTSuV2), grouped into the genus *Iotatorquevirus* (International Committee for Taxonomy of Viruses).

TTSuVs have been found worldwide in pigs. Prevalence rates tend to increase by age, and can be as high as 100% (2). Also, it has been shown virus DNA loads, for both TTSuV species (TTSuVs), increase with age (3). The potential link of TTSuVs with disease occurrence is under debate. TTSuV1 infection has been suggested to act as a disease triggering in co-infection with *Porcine circovirus type 2* (PCV2), the causative agent of postweaning multisystemic wasting syndrome (PMWS) (4). In addition, higher TTSuV2 prevalence has been found in pigs suffering from PMWS (2).

The objective of the study was to describe the kinetics of viral DNA loads of both TTSuVs in serum of PMWS affected pigs and age-matched, healthy counterparts.

Materials and methods

Clinically healthy (H) pigs (n=17) and PMWS affected pigs (n=18) were included in this study, based on body condition, absence/presence of clinical signs, histopathological findings and PCV2 amount in lymphoid tissues measured by *in situ* hybridization (ISH) (5). These pigs were longitudinally monitored until PMWS development and sera was available at 1, 3, 7 and 11 weeks of age, plus at the age of PMWS presentation (around 15 weeks of age, N), in which sick animals and H age-matched pigs were euthanized and necropsied.

Serum of these pigs were tested by a real time quantitative PCR (qPCR) using specific primers to detect each TTSuVs (6). Chi square test and ANOVA was carried out to asses differences between groups.

Results

Viral DNA loads increased with age until 11 weeks of age and then declined, except for TTSuV2 in PMWS affected pigs; in these animals, maximum viral loads were reached at necropsy week (N), TTSuV2 loads were significantly lower in H age-matched pigs at the week of necropsy ($p < 0.05$). Differences between groups were not evident in any other sampling point (figure 1).

Prevalence were not significantly different for any TTSuVs, although, in case of TTSuV2, in week 11 and N a tendency ($p < 0.1$) was observed when comparing H against PMWS affected pigs.

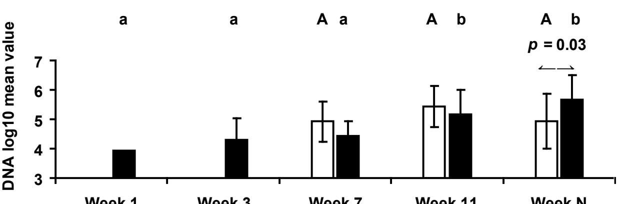

Figure 1: TTSuV2 viral loads dynamics in H (white bars) and PMWS (black bars) affected pigs. Mean viral loads, standard deviation and P-values are shown. Significant differences between ages within H (capital letters) or PMWS (case letters) represented.

Discussion

The present results show the *in vivo* dynamics of TTSuVs infection in H and PMWS affected pigs. It was observed that TTSuV2 viral DNA loads increased over time until last sampling in PMWS affected pigs, while this was not the case for H pigs. Apparently, TTSuV2 may benefit of the disease status by increasing viral release or replication, while this would not be the case for TTSuV1, apparently. These observations might support others from Torque teno viruses (TTVs) in humans, since some of the viral species seem to be more related with disease occurrence than others (2).

Noteworthy, inmunosupression can induce an increase in TTV viral load in human beings (7). Taking into account the immunosuppressive nature of PMWS (8), it would not be surprising a similar effect of at least TTSuV2 in pigs.

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This work was funded by grants AGL2006-02778/GAN, TRT2006-00018 and CONSOLIDER-PORCIVIR CSD2006-00007.

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TORQUE TENO SUS VIRUS 2 (TTSUV2) LOAD IN SERUM OF PORCINE CIRCOVIRUS TYPE 2 (PCV2) VACCINATED AND NON-VACCINATED PIGS

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Introduction

Anelloviruses are small, non enveloped viruses with circular, single stranded DNA genome (1). In swine, two species with substantial sequence heterogeneity have been identified (2): *Torque teno sus virus 1* (TTSuV1) and 2 (TTSuV2), both grouped into the genus *Iotatorquevirus.*

TTSuVs have been linked with postweaning multisytemic wasting disease (PMWS) (3,4,5), a *porcine circovirus type 2* (PCV2) disease. However, this association with disease seem to be more consistent for TTSuV2. It would appear that PMWS occurrence may up-regulate TTSuV2 replication, but the pathogenicity of TTSuVs is still under debate.

Taking into account that PCV2 vaccines are able to efficiently counteract PMWS under field conditions, it was hypothesized that TTSuV replication in a population of PCV2 vaccinated pigs should be more limited than in a group of non-vaccinated animals. Therefore, the aim of this study was to determine whether PCV2 vaccination was effective in reducing TTSuV2 load, quantified by means of a quantitative PCR (qPCR) method.

Materials and methods

One hundred and fifty pigs were chosen for this experiment. Half of them (75 pigs) received an intramuscular commercial PCV2 one dose vaccine (V) at 3 weeks of age, while the other 75 pigs received PBS as placebo (NV). Pigs were longitudinally monitored and blood was taken at 8, 16 and 21 weeks of life. All DNA samples were tested by a real time quantitative PCR (qPCR) using specific primers to detect TTSuV2 (5).

For the statistical study, four groups were considered. Each group of 75 pigs was sub-divided into two groups: pigs PCV2 positive (PCV2+) and PCV2 negative (PCV2-). Such classification resulted from the application of a standard PCR method to detect PCV2 genome (6). Chi square test and ANOVA were carried out to assess differences between groups.

Results

No statistical differences were observed in viral load or prevalence when PCV2 vaccinated pigs were compared with non-vaccinated ones. Also, no significant differences were found when the groups of animals were sub-divided between those with PCV2 PCR positive and negative results (table 1). The higher TTSuV2 load in serum as well as higher prevalence was observed at the end of the field study.

Table 1. Average values for TTSuV2 load (expressed as log10 TTSuV2 DNA copies/ml of serum).

Group / Week		Week 8	Week 16	Week 21
NV (PCV2-)	Mean	5,08	4,63	5,46
	SD	0,66	0,72	0,60
V (PCV2-)	Mean	4,93	4,72	5,46
	SD	0,59	0,59	0,73
NV (PCV2+)	Mean	4,65	4,75	5,40
	SD	0,72	0,67	0,70
V (PCV2+)	Mean	4,43	4,53	5,32
	SD	0,35	0,50	0,57

Discussion

PMWS affected pigs seem to have higher TTSuV2 loads than healthy animals (5). Therefore, the present study explored the potential effect of PCV2 vaccination on TTSuV2 amount in serum. However, data did not show differences in TTSuV2 load between pigs vaccinated or not against PCV2.

Most of the pigs in the present study developed a PCV2 subclinical infection but not PMWS (data not shown). Lack of difference in TTSuV2 load between groups NV (PCV2-) and NV (PCV2+) suggests that a subclinical PCV2 infection is probably not enough to up-regulate TTSuV2 replication as it apparently happens in those pigs having clinical disease. Since PMWS affected pigs are claimed to suffer from immunosuppression (7), it is likely that such dysfunction of the immune system contributes to up-regulation of TTSuV2.

Acknowledgements

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TORQUE TENO SUS VIRUS TYPE 1 (TTSUV1) AND TYPE 2 (TTSUV2) IN SITU HYBRIDIZATION ON FORMALIN-FIXED, PARAFFIN EMBEDDED TISSUES

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Introduction

Torque teno viruses (TTV) are non-enveloped, circular, single stranded DNA viruses that may infect human, non-primate and domestic species. In swine, two specific TTV species (TTSuV1 and TTSuV2) have been described. Even though the infection in pigs is considered non-pathogenic, TTSuVs have been somehow linked with porcine circovirus diseases like postweaning multisystemic wasting syndrome (PMWS) and a porcine dermatitis and nephropathy syndrome (PDNS)-like syndrome (1,2,3,4).

An *in situ* hybridization (ISH) method, using the tyramine signal amplification system, was set up to detect TTSuV1 and TTSuV2 DNA in formalin-fixed, paraffin-embedded transfected PK-15 cells (5). Cells were transfected in serial dilutions as to determine a detection limit for the ISH technique (5). In the current work, the method was applied on formalin-fixed paraffin embedded tissues.

The aim of this work was to evaluate the detection limit and the application of the ISH technique on tissues from healthy and PMWS affected pigs.

Materials and methods

Healthy and PMWS pigs were selected by means of TTSuV1 and TTSuV2 loads in serum measured by real time quantitative PCR (RT-qPCR) method (4). A total of 20 pigs were selected: 10 healthy animals and 10 PMWS affected ones (5 with the highest viral load for TTSuV1 and 5 with the highest viral load for TTSuV2 for each group).

ISH was performed on formalin-fixed, paraffin-embedded tissues (lymph nodes and lung) of the selected animals. , Cloned TTSuVs viral genomic DNAs were labelled with digoxigenin (DIG) separately to be used as specific probes in hybridization. Negative and positive controls based on TTSuV1 and TTSuV2 transfected PK-15 cells were used (5). The ISH method was based on previously described protocols (6,7).

Besides, fresh tissues (lymph nodes and lung) from the same animals were tested by RT-qPCR.

Results

No specific labelling of TTSuV1 and TTSuV2 DNA was observed in lung and lymph nodes tissues from any of the healthy or PMWS affected pigs using the ISH technique. Positive controls (transfected cells) displayed the corresponding positive cytoplasmic cell labelling using both TTSuV probes.

Results of RT-qPCR for both viral species on fresh lung and lymph nodes tissues are presented in table 1.

Table 1. TTSuV1 and TTSuV2 viral load ranges in serum, lung and lymph node from healthy and PMWS affected pigs and ISH detection limits. Values are expressed by molecules per µg of total DNA, except for serum as molecules per ml of serum.

	TTSuV1		TTSuV2	
	Healthy	PMWS	Healthy	PMWS
Serum viral load	10^4-10^6	10^5-10^7	10^5-10^7	10^6-10^{10}
Tissues viral load	10^2-10^4	10^4-10^5	10^2-10^4	10^4-10^6
ISH detection limit	10^8		10^7	

Discussion

The present ISH technique was initially developed on PK-15 cells transfected witht TTSuV DNAs and subsequently formalin-fixed and paraffin-embedded (5). This strategy was chosen to ensure that the technique was not affected by the process of fixation and embedding in paraffin. Therefore, the negative results of this ISH applied in tissues of healthy and diseased animals seem to be due to a non-sufficient amount of both TTSuVs present in the examined animals, ruling out technical limitations.

These results would support the sub-clinical nature of TTSuV infections and, even their loads seems to be up-regulated in the context of porcine circovirus diseases, the final amount of virus in tissues is still too low to be detected by the developed ISH, at least in lymph nodes and lung.

Acknowledgements

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PHYLOGENETIC CHARACTERIZATION OF HEPATITIS E VIRUS (HEV) ISOLATES FROM CROATIA

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Introduction

The hepatitis E virus (HEV) is a small non-enveloped RNA virus belonging to the genus *Hepevirus, Hepaviridae* family. Pig may be an animal reservoir host for HEV, and therefore its zoonotic and xenozoonotic potential is a significant health concern. The first swine HEV isolate that was genetically and serologically related to human HEV strains was detected in swine herds in the US [1]. According to a previous study, a high prevalence to HEV antibodies has been demonstrated in the Croatian pig population [2]. It is well known that European swine HEV belongs to genotype 3 [3]. The main objective of the present study was phylogenetic characterization of HEV isolates from Croatian pig production units.

Materials and methods

All together 60 pig serum samples from three different farrow-to-finish pig herds from different age group of pigs and bile from 37 pigs that died from different clinical pathological conditions were collected. Presence of HEV genome was detected by nested RT-PCR as previously described [4,5]. PCR products were purified and fully sequenced using the ABI Prism BigDye™ Terminator Cycle Sequencing kit (Applied Biosystems). Sequencing reactions were analysed on an ABI 3100 automatic DNA sequencer (Applied Biosystems). Sequences were assembled using the SeqMan II software (DNASTAR, Madison WI, USA). Multiple alignments of the nucleotide sequences were performed using Clustal X. Construction of a phylogenetic tree was performed using the ClustalW program using a neighbour-joining algorithm and the Kimura two-parameter model in the MEGA software.

Results

In this study, 8 of 60 swine serum samples and 3 of 37 swine bile samples were positive for HEV. We were able to identify 6 sequences HEV ORF2 encoding region. Sequences are deposited in the GenBank (HQ591362, HQ591363 and HQ591364).

Discussion

Two sequences (HQ591363 and HQ591364), both grouped in subgroup "j", shared 90% nucleotide sequence identities with HEV isolate from swine from Canada (AY115488).

The swine sequence (HQ591362) from the present study shared 92% nucleotide sequence identity with HEV isolate swJB-D4 from swine faeces from Japan (AB471006), and 92% with swine serum HEV isolate swJ8-12 from Japan (AB094237). This Croatian isolate of swine origin belongs to genotype 3, and the "e" subgroup of swine HEV form a separate branch, most related to the Japanese sequences and several European and Hungarian virus isolates [5]. Finally, all three identified Croatian swine HEV serotypes in this study originate from animals 3 to 5 months of age from different swine herds and geographical regions.

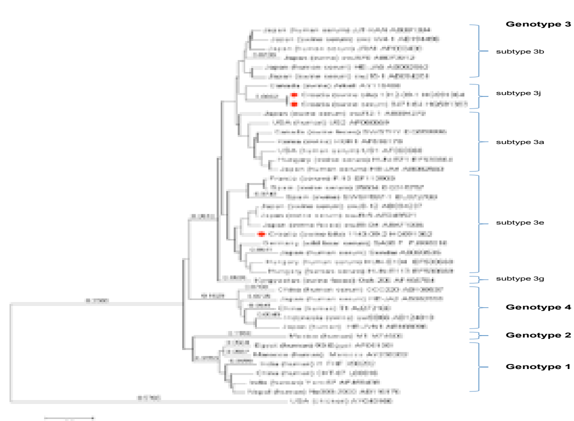

Fig 1.Neighbor-joining analysis of partial HEV ORF2 sequences showing the phylogenetic relationship between Croatian swine HEV isolates and other related isolates from the GenBank. GenBank accession numbers are indicated for each sequence.

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SCREENING OF RESERVOIRS FOR HEPATITIS E VIRUS

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Introduction

Hepatitis E virus (HEV) is a small non-enveloped positive sense RNA virus that causes acute hepatitis E in humans in developing countries of Asia and Africa, but is also seen sporadically in the industrialized world. HEV is divided into four genotypes where genotype 1 and 2 only infects humans; genotype 4 is found in human and swine; genotype 3 has been found in a wider host range (including human and swine). More genotypes have been characterized including a rat and an avian HEV variant. The avian HEV only resembles Genotype 1-4 50% at the nucleotide level. In a recent study HEV antibodies were found in ~91% of Danish pig herds with the viral RNA present in ~50% of pigs aged 4-22 weeks (1). It is now recognized that HEV is a zoonotic disease with swine being the primary reservoir. In the present study several animal species were investigated for the possibility of an alternative reservoir. Samples from dog, cats, deer, pheasant, mink and rabbits for HEV were screened using PCR.

Materials and methods

RNA purification: A 10% suspension of each sample was made in PBS. From this 450 ul was homogenized using a Tissuelyzer (Qiagen) for 20 sec at 15 Hz. The samples were purified using Virus/bacteria kit on the QIAsymphony (Complex 200, Qiagen).

Nested PCR: The screening of samples was performed by a nested PCR (nPCR) assay developed by Reimar Johne et al. (2010) (2). The assay targets a conserved region in genotype 1-4 as well as avian HEV and rat HEV. This should ensure that distantly related HEV strains are also detected. All samples were run on 2% E-gels (Invitrogen) for 30 minutes post amplification.

Real Time PCR: An assay targeting a highly conserved region in ORF2 specific for genotype 1-4 was also used[1]. The assay takes advantage of the PriProET chemistry.

Cloning: The PCR products from the nested PCR were purified from the gel using Roche High Pure PCR product purification kit as described in the protocol. A TOPO TA cloning kit using the pCR4 vector (Invitrogen) was used for cloning in *E. coli* TOP10 competent cells.

Results

A total of 124 samples have been screened with the nPCR assay (77 mink, 5 cats, 36 dogs, 2 deers and 4 pheasants). From these 124 samples one mink sample was found positive using the nPCR. This is shown in Figure 1; where the mink sample (lane 6) has the same band pattern and size as the positive control (lane 1). This sample was also tested using the real time PCR assay and was found to be negative. This result indicates that a novel HEV has been discovered in mink. The mink from which this sample was recovered showed severe damage in the liver but it has not been possible to determine if this damage was due to the HEV infection. Currently progress is being made to clone the nPCR products from the positive sample and sequencing is planned. Furthermore, screening of more samples from the species listed above, with the inclusion of rabbit samples, will be performed.

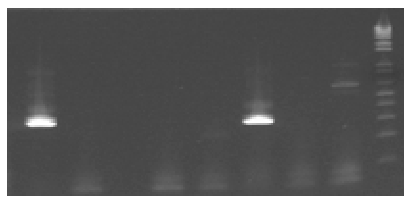

Figure 1. Gel showing the **r**esults of nPCR: Lane 1: Positive control (known genotype 3 sample from swine); lane 2: Water, lane 3: Blank; lane 4-8: Mink samples; lane 9: DNA ladder (100bp)

Discussion

This is the first time, to our knowledge, that HEV has been detected in mink. Especially mink were of interest prior to testing, as they in a period from 2007 to 2008, in some parts of Denmark, were fed with pig waste products from slaughterhouses. The negative result of the real time PCR assay suggests that this strain has not been transmitted from pigs as it is not genotype 3 or 4. Likewise from the real time PCR this mink HEV does not belong to genotype 1 or 2 either. Sequence data is needed to clarify the phylogenetic relationship of this HEV. These investigations are currently being performed along with screening of more samples. These results indicate that swine is not the sole reservoir for HEV in Denmark.

Acknowledgements

Ministry for food, agriculture and fisheries for funding of this project.

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IMPORTANCE OF DOMESTIC PIGS AS A RESERVOIR FOR AUTOCHTHONOUS HEPATITIS E IN FRANCE

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Introduction

The hepatitis E virus (HEV) is responsible for epidemics of viral hepatitis, in many countries of Asia and Africa. In most cases acute hepatitis, similar to hepatitis A, is observed but in 1-2% of cases, infection leads to fatal fulminant hepatitis. During these outbreaks, the HEV is mainly transmitted through contaminated water or soiled food. HEV is also responsible for sporadic cases in non-endemic regions such as the USA, Europe and Japan. In most cases the routes of contamination remain unknown. Unlike other hepatitis viruses, human is not the only natural host of HEV and its presence in many animal species, especially pigs, suggests that it is a zoonotic agent. Several cases of direct transmission of HEV from animals to humans have been reported in Japan after eating undercooked food contaminated with HEV.

In France, the National Reference Centre of HEV*, reports every year more than two hundred cases of autochthonous hepatitis E, non-associated with travel to endemic areas. The origin of these indigenous cases is not well characterized, but several cases have been associated with the consumption of raw pig liver sausage. To determine whether the porcine reservoir could be the cause of human cases, a prevalence survey was conducted in the domestic swine population.

Materials and methods

One hundred eighty-six farms were sampled throughout the national territory by making a random selection of these farms through a draw of batches of pigs at the slaughterhouse. A representative sample of pigs from selected batches was analyzed in terms of serological and virological prevalence (serum sample and matched liver). During this survey 6565 sera and 3715 livers were analysed leading to the estimate of seroprevalence and virological prevalence at the individual and farm levels. Seroprevalence data were corrected for sensitivity and specificity of the test used (1).

Results

The results showed that seroprevalence of HEV at the farm level was 65.3% [95% CI 56.6 - 73.9] and that 30.7% [95% CI 23.8 - 37.6] of animals had antibodies to HEV. The results have shown a virological prevalence of 4.0% [95% CI 2.1 - 5.9] in livers entering the food chain. These results also showed a strong correlation between virological and serological results: The probability for a batch of pigs to contain at least one positive liver was greatly increased when the within-farm seroprevalence was high (> 25%) (OR = 6.7 [2.1 - 21.6]). This indicates that the likelihood of contamination of livers at slaughtering is conditioned by the within-herd dynamics of infection. It should be noted that there is also an effect of the geographical location of farms on seroprevalence and virological prevalence of HEV. In addition, a phylogenetic analysis of hepatitis E virus sequences found in livers was carried out in comparison with the sequences isolated from humans. This analysis of 306 nt of ORF2 showed that the same subtypes were found in both species: 3f, 3e and 3c and there is no cluster according to the species from which the sequence has been isolated (human or pig liver). One of the amplified sequences in liver had 99.3% homology of nucleotides amplified with a strain in human, which suggests very strongly that zoonotic transmission may have occurred.

Discussion

This survey was performed to characterize the level of contamination of pig livestock regarding the HEV. These data will allow achieving a quantitative risk assessment of exposure to HEV in contact with the reservoir or through consumption of pork products. These results will contribute to define the level of surveillance to limit zoonotic transmission of this virus.

Acknowledgements

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PRELIMINARY VALIDATION DATA OF A COMMERCIAL MULTI-SPECIES ELISA KIT FOR DETECTION OF ANTI-HEPATITIS E VIRUS (HEV) ANTIBODIES IN SWINE

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Introduction

Hepatitis E is caused by infection by the hepatitis E virus (HEV), a non-enveloped, positive-sense, single-stranded RNA virus affecting humans, cows, sheep, goats and rodents. A waterborne disease, contaminated water or food supplies have been implicated in major HEV outbreaks.

In industrialized countries, however, many hepatitis E cases are of undetermined origin. Some cases may be linked to zoonotic transmission of HEV from pigs and wild boars (domestic animals have been reported as a reservoir for the hepatitis E virus, with some surveys showing infection rates exceeding 95% among domestic pigs(1).) Transmission after consumption of uncooked meat has also been reported (2).

The rate of HEV-transmission to humans via contaminated food and the public health consequences, particularly for pregnant woman, are still unclear (3).

In non-endemic zones, isolated strains from humans and pigs belong to the same genotype (mainly 3 and 4). Commercially available kits to detect anti-HEV antibodies only exist for humans, and are based on genotype 2 capsid antigen (ORF2) or peptides (4).

In this context, ID VET has developed the first commercial multi-species indirect ELISA based on a recombinant capsid protein of genotype 3 HEV, ID Screen® HEV Indirect Multi Species.

Materials and methods

Test procedure :

A recombinant capsid protein from genotype 3 HEV is expressed in baculovirus and affinity purified. Plates are coated at the optimal concentration. Controls and sera to be tested are diluted at 1:20 in serum dilution buffer and incubated 45 minutes at room temperature (RT). After washes, a multi-species conjugate* is added for 30 minutes at RT. Plates are washed, and the substrate (TMB) is added. After 15 minutes the reaction is blocked by adding the stop solution, and optical densities read at 450 nm.

* for pigs, wild-boars, ruminants and humans. Contact IDVET for more information.

Results

Specificity : 42 sera from SPF pigs were tested. All sera tested were found negative, giving a measured specificity of 100% (ICI_{95} 99,0 – 100%).

Sensitivity: 176 pig sera collected from two herds in France with known HEV infections. A seroprevalence of 48 and 62% was observed. A sample set of mini pig and wild boar sera from experimental HEV (genotype 3) challenge studies were tested (FLI, Riems). The panel included negative control sera as well as defined low and high positive sera of 10 pigs at 38 days after infection. Seroconversion profiles were analysed and presented.

Analytical sensitivity: As no internal standard exists, analytical sensitivity is tested using an internal weak positive standard (a strong positive serum diluted in a negative one) proposed by ID VET.

Repeatability: Repeatability was evaluated by calculating the coefficient of variation (CV) for 96 repetitions of the internal weak positive control, and 96 repetitions of the positive control of the kit. The CVs obtained were found to be between 4 and 9%.

Discussion

The ID Screen® HEV Indirect Multi Species ELISA is the first genotype 3 ELISA which is commercially available. This study indicates that the test has high specificity and sensitivity. The fact that it is based on a recombinant capsid protein of genotype 3 should improve sensitivity in swine populations, where circulation of HEV is a growing concern. Both this ELISA and a commercial genotype 1 ELISA (for human diagnostics) have shown similar sensitivity on 10 sera from experimentally-infected wild boars and mini pigs. The data, however, must be completed with results from seroconversion experiments and studies on free pigs. These projects are underway and will be presented on the poster.

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HIGH THROUGHPUT DETECTION OF HEPATITIS E VIRAL RNA IN FAECES OF AUSTRIAN DOMESTIC PIGS

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Introduction

Hepatitis E virus (HEV) is a small, non-enveloped, single stranded positive sense RNA virus, and member of the Family *Hepeviridae*. The virus is transmitted by the fecal-oral route and is a frequent cause of hepatitis in humans, especially in developing countries. HEV infection may even lead to death in pregnant women.

The presence of HEV RNA in faeces, serum and organ samples and the high seroprevalence of HEV antibodies in swine from many Asian, North-American (1) and some European countries (2) was already reported, indicating worldwide distribution of HEV in swine herds.

HEV was detected and characterised for the first time in samples from Austrian domestic pigs in 2010 (3). The HEV strains identified all belong to genotype 3, which is a potential zoonotic pathogen. Due to these findings, a novel sensitive one-step real-time RT-PCR (RT-qPCR) was established for the detection of HEV in faeces and organ samples. This method enables screening for HEV in domestic pigs in the most intensive swine producing regions of Austria.

Material & Methods

For our investigation, faeces and liver samples from 299 randomly selected piglets, ranging from 1 to 3 months of age, were collected from 3 regions of Austria which have a high density of domestic swine.

RNA extraction was performed on a fully automated platform with two different extraction kits.

For HEV detection, an ORF3 gene based HEV RT-qPCR (3) was was employed. The dynamic range and the sensitivity of the HEV RT-qPCR were determined by testing a serial dilution of *in vitro* transcribed RNA, as well as a number of positive samples. For specificity testing, a number of common viral and bacterial pathogens of swine were included.

Results

From a total of 299 tested pigs, 47 pigs (16%) were positive by RT-qPCR. Cq values ranged from 19,65 – 37,12 in liver and 21,0 – 36,66 in faces. Among all positive animals, HEV was detectable in 22 (47%) pigs in both, liver and faeces. Four of these animals came from Lower Austria, 4 from Burgenland and 14 from Styria. In the other RT-qPCR positive pigs, HEV was only found in liver (21 samples) or faeces alone (4 samples). A summary of HEV RNA positive pigs is shown in Table 1.

Table 1. *HEV detection in liver and faeces of pigs submitted from different Austrian regions*

Region	HEV RNA detection				
	pos. liver and faeces	pos. liver	pos. faeces	neg. samples	Total
Lower Austria	4	2	0	59	65
Burgenland	4	0	2	28	34
Styria	14	19	2	165	200
Total	22	21	4	252	299

(Note: pos.=positive, neg.=negative)

Discussion

HEV of genotype 3 has been recently described in Austrian domestic pigs (3). Our findings clearly demonstrate that a combination of fully automated RNA extraction and one-step RT-qPCR successfully determined the shedding of HEV in the faeces of 1-3 months old piglets.

According to the results obtained from our big study, 15% of the Austrian domestic pig population is infected with HEV before slaughtering, which is much lower than the prevalence reported by other countries (1, 4), where only 43 (1) and 49 (4) animals were tested, respectively. Furthermore, the results also show that the virus is not always excreted in faecesof infected pigs, as the virus is more often found in liver samples, which would be disregarded in *pre-mortem* assays where only faeces should be tested. Further assay validation using endogenous reference gene detection in a duplex RT-qPCR will be performed to avoid false negative results due to PCR inhibition and/or sample degradation.

Acknowledgements

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COMPLETE NUCLEOTIDE AND AMINO ACID SEQUENCES OF PORCINE KOBUVIRUS ISOLATED FROM PIG

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Introduction

The genus *Kobuvirus* is contained within the family *Picornaviridae,* which comprises small, non-enveloped viruses with single strand, positive-sense genomic RNA. Porcine kobuvirus were isolated from pig fecal samples in Hungary in 2007. To investigate the complete nucleotide and amino acid sequences of the Korean porcine kobuvirus, the 61WA13 strain of collected 43 strains from three pig farms with good breeding facilities in three provinces from March to May of 2010 was used this study and was analysed with two strains (JY-2010a/CHN and S-1-HUN/2007/Hungary) isolated from China and Hungary.

Materials and methods

The sequences were aligned and analyzed using the ClustalW 1.8x program and the Mega 4 program. The predict RNA secondary structures from sequence was using the RNAstructure program.

Results

The amino acid sequences between 63WA13 and S-1-HUN strains isolate from Korea and Hungary country, respectively, were changed in 11 position sites for three proteins (L, VP0, and 2A), five position sites for three proteins (VP1, 2B and 2C), while the VP3, 3A, 3B, 3C, and 3D proteins displayed changes in six, four, zero, three, and nine position sites, respectively (Fig 1).

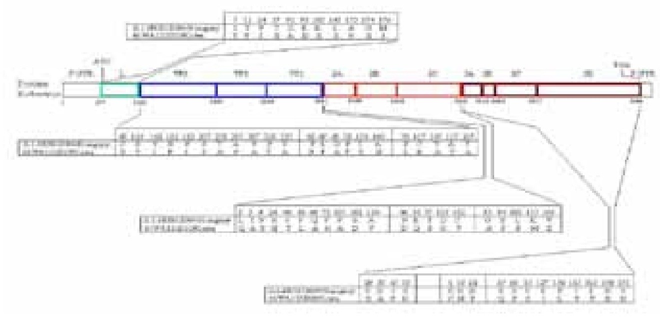

Fig. 1. Mutation changes in amino acid sequences of two porcine kobuvirus strains.

The predicted N-terminal cleavage sites among proteins were conserved at all cleavage sites except 3C/3D proteins of three strains, whereas the cleavage site of the 3C/3D proteins of 63WA13 strain was Q/S, but the remaining strains (S-1-HUN and JY-2010a) displayed Q/C amino acid sequences.

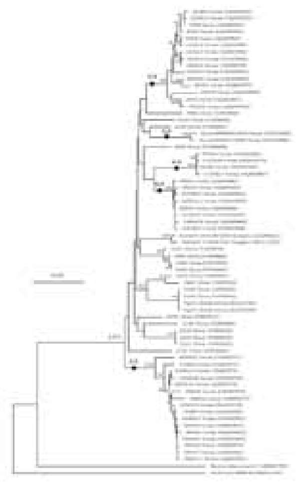

Fig. 2.
Phylogenetic tree of porcine kobuvirus strains. The tree was constructed using the Neighbor-joining method and shows the phylogenetic relationship between the 67 global porcine kobuvirus strains and two other strains (bovine kobuvirus and Aichivirus). Bootstrap percentages are shown above those branches that are supported in at least 60% of the 1,000 replicates.

The five Korean lineages, G1 (Okchen), G2 (GU723955 and GU723956), G3 and G4 (Dangjin), and G5 (Wongju), had high levels of bootstrap support (99% or 100%) (Fig. 2). The 61WA13 strain isolated from pig farm in Wongju province was included in the G5 group.

Discussion

It is likely that porcine kobuvirus is not restricted geographically and is widely distributed on pig farms worldwide. The secondary structure of the extreme 5'untranslated region (576 nt) is very similar to those of three porcine kobuvirus (63WA13, S-1-HUN and JY-2010a). The major differences between kobuviruses and other pocornaviruses are found in the coding region of the L protein, the absence of a VP0 cleavage site and a distinct form of the 2A protein (1). Genetic identity of amino acid sequences on the coding region of the JY-2010a and 61WA13 strain maintains lower percent than that of the S-1-HUN strain except VP3, 2C, and 3C proteins. Although the China strain is close in its geographical relationship and analysis of phylogenic tree by conserved P3 protein sequences, the 61WA13 strain isolated from South Korea was demonstrated as similar with the S-1-HUN strain based on the analysis of genomic sequences.

Acknowledgements

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HN RECOMBINANT PROTEIN OBTAINED FROM PORCINE RUBULAVIRUS AND ITS EVALUATION AS AN IMMUNOGEN FOR THE BLUE EYE DISEASE CONTROL IN PIGS

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Introduction

The Blue Eye disease (BED) is considered endemic in Mexico and exotic for other countries, which represents a limit for pig commercial exports. The disease is caused by Porcine Rubulavirus (PoRV) and is characterized by causing infertility, nervous alterations and respiratory problems in adult pigs; in some cases a corneal opacity is observed at all ages animals. The object of this study was to obtain the main PoRV protein (HN) that stimulates the immune system as well as, the evaluation of humoral immune response induced on laboratory animals.

Materials and methods

The reference strain PoRv-LPVM-84 was used to clone the gene that codifies for the HN Porcine Rubulavirus protein, with the pDual GC Expression Vector System.

DH5α cells were transformed in order to obtain the clone. The cell colonies were evaluated by PCR and observed at 2% agarose gels with ethidium bromide. The plasmid was purified and KRX competitive cells were transformed for the HN protein expression, which was purified by Histidine column and evaluated by Western Blot tests with an Anti-HN specific monoclonal antibody obtained in mouse (first antibody) and Anti-IgG Anti-mouse conjugated with peroxidase as a second antibody. Once the recombinant protein was purified, 21 days old CD1 mice were inoculated, applying 10μg of the HN recombinant protein, split in two doses, by subcutaneous route. The ELISA test was used to verify the humoral immune response.

Results

From the transformed colonies, 15 clones were selected, where the insert of the HN fragment represented 100% accuracy as evaluated by PCR. The HN expression capability was demonstrated by Western Blot, revealing the presence of a 60 kDa protein with the specific monoclonal antibodies against the HN protein. The humoral response in immunized mice using ELISA test, was detected from the seventh day post-inoculation, which showed optical densities between 0.8 – 1.0 DO, as in negative control mice were from 0.005-0.01 DO.

Discussion

The recombinant product obtained from the gen that codifies for the HN protein from PoRV shown to induce an specific humoral response. This technological development represents a useful advance for the future recombinant vaccine, as well as, for the diagnostic methodology control design for Blue Eye Disease in Mexico.

Acknowledgements

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QUANTIFICATION OF HUMORAL IMMUNE RESPONSE TO PORCINE RUBULAVIRUS INFECTION IN BOAR

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Introduction

Blue eye disease (BED) is an endemic disease in Mexico, in which porcine rubulavirus (PoRv), a member of *Paramyxoviridae* family, is its etiologic agent (1). Different works report BED to be the cause of orchitis, epididymitis and decrease of seminal quality in boars (2,3). Importantly, viral RNA persistence and infective virus in testis and epididymis has been documented in both short and long term infections (2,4,5). Moreover, despite humoral immune response in serum has been reported by a number of conventional tests (4,6) and that specific antibodies in seminal plasma have been identified in PRRSV infection (7) IgG quantification for PoRv has never been investigated. Therefore the aim of this study was measure the amount of serum IgG and seminal plasma IgA, IgM and total and specific IgG PoRv infection.

Materials and methods

Sera (*n*=83) and seminal plasma (*n*=73) were collected from nine boars experimentally infected with the PAC-3 strain(5). A capture ELISA was performed for total quantification of immunoglobulins in seminal plasma Bethyl Laboratories, Inc.), whereas for of IgA, IgM and IgG in seminal plasma and serum IgG a quantitative indirect ELISA was used. Tests were performed using purified PoRv adjusted to a protein concentration of 1.3μg/100μl. In both cases the immunoglobulins were quantified (μg/ml) using a standard curve and the optical density was obtained at 450nm.

Results

Serum IgG concentration against PoRv increased after experimental infection, retaining a high concentration until the end of the experiment (Fig 1). Total immunoglobulins in seminal plasma showed a predominant concentration of IgA, which decreased significantly by week four postinfection while level of IgG reached its plateau at week 6. Consistently, IgM had an average concentration of 1.5 ± 0.28 μg/ml, being the least abundant isotype in seminal plasma (Fig 2). Specific IgA was only detected at week 6 (0.013 μg/ml) and 12 (0.003 μg/ml) in one boar, whereas only one boar showed low concentrations of specific IgG at 4, 6, 8, 10, 12 and 14 weeks postinfection (0.15, 0.06, 0.01, 0,008 and 0,008 μg/ml, respectively). No specific IgM detected.

Figure 1. Quantification of seric IgG against PoRv in experimentally infected boars.

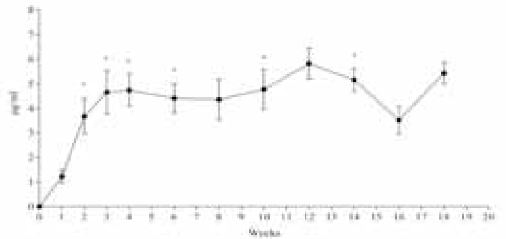
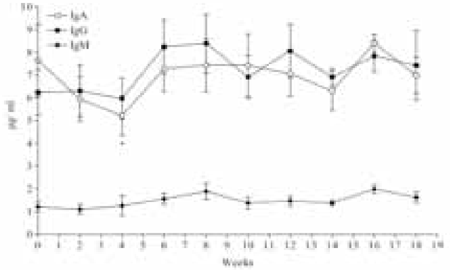


Figure 2. Quantification of total immunoglubulins in seminal plasma of experimentally PoRv infected boars.



Discussion

The curve of IgG concentration obtained in this study is consistant with that observed in tests carried out with conventional probes (6) with the difference that the amount was quantified in micrograms of immunoglobulin. Intrestingly, our quantification of total immunoglobulin differs from previously published results (8), since the predominant isotype was IgA. In conclusion, the detection of specific IgA and IgG against PoRv, although not in all boars (7), represents very interesting finding which encourages us to further investigate on this topic.

Acknowledgements

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P.246
LEUKOSPERMIA AND SEMINAL QUALITY AFTER EXPERIMENTAL INFECTION WITH PORCINE RUBULAVIRUS

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Introduction

The porcine rubulavirus (PoRV) can lead to the formation of granulomas in the epididymis and the degeneration of the epithelium epididymal in boars experimentally infected (1, 2). This lead to the decline of the seminal quality as well as the presence of white blood cells, which increase the formation of reactive species of oxygen (ROS) (3)). ROS damage sperm acrosome membrane. The leukocytes analysis presence, in boar semen, is not a routine test for quality control of seminal doses, however, in human is performed with strict care, because of its association with low fertility patients. The present study evaluated the presence of leukocytes in boar semen its effect on the membrane acrosomal and semen quality in experimentally PoRV infected boars.

Materials and methods

Nine boars York-landrace 12 months old reproductive diseases free were used. Two stages were evaluated: before-infection (B-in) and after-infection (A-in). Boars were inoculated intranasal with 5 mL 10^5TCID$_{50}$/mL of strain PAC-3 Jalisco/1992 of the PoRV. The unfractionated ejaculates were evaluated: volume (VmL), sperm concentration (spz. x10^{-1}), motility progressive (%MTP), abnormalities (%Anor), acrosomal membrane A-in from the 4 week with the lectin *Triticum vulgaris* (4). Acrosomal membrane assessment: membrane integrated (MAI), damaged (MAD) and acrosomal reaction (MAR). The leukocytes count (L x10^3mL) was conducted in two phase, using a Neubauer counting camera (5), and stained slides with Wright solution. Data were analyzed as repeated measures, using the PROC Mixed of SAS (SAS INST. INC., Cary, NC). The model included the fixed effects of treatment, week and treatment x week interaction and the random effect of board.

Results

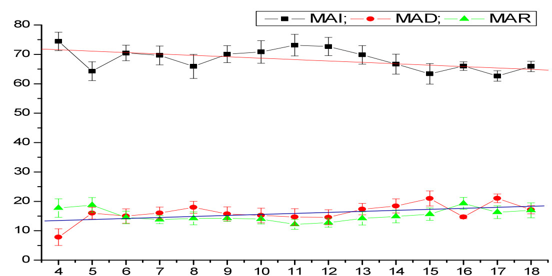
Volume, concentration spz, MPT and abnormalities showed significant differences ($P<0.05$). Acrosomal evaluation showed negative trend for MAI. In contrast to MAD and MAR the trend was positive. The presence of leukocytes showed significant differences ($P<0.05$) between stages.

Discussion

The production and semen quality of boars during Phase I was similar to healthy boars and weekly work rhythm (6). However, during A-in, sperm production decrease as well as the leukocytes presence beginning at eight days after-infection ($P<0.05$). The production and semen quality of boars during B-in was similar to healthy boars and weekly work rhythm (6). However, during A-in, sperm production decrease 11.47% (Vml), 4.47% (spz) was more evident, as well as the leukocytes presence beginning at eight days after-infection ($P<0.05$).

Table 1. Least square means (± SEM) for semen quality before and after infection. [a,b] Means within a row without a common superscript differ ($P>0.05$).

	B- in	A-in
VmL	172.83^a ± 6.49	153.10^b ± 3.84
Spz. x10^{-1}	312.33^a ± 23.07	298.34^b ± 11.55
% MTP	85.37^a ± 0.97	81.85^b ± 0.54
Abn%	4.89^a ± 1.03	5.76^b ± 0.71
L x10^3 mL	--------------	30.2^b ± 21.66



Although abnormalities differences ($P<0.05$) were lower than those previous reported (2). Leukocytes effect increases acrosomal membrane damage as a consequence of ROS increase (3). There was a significant increase on weekly leukocytes, the highest value occur at the eighth week, and gradually decline, reaching at a level of 20 leukocyte x10^3 ml, holding constant these values at last five weeks of the experimental study.

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RE-EMERGING TRANSMISSIBLE GASTROENTERITIS IN PIGS

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Introduction

Transmissible gastroenteritis (TGE) of pigs is an enteric disease caused by a porcine coronavirus. It can affect animals of any age but the clinical signs are less pronounced in adults. Piglets without maternal immunity, especially during the first week of life develop devastating intestinal infections resulting in vomiting, diarrhea and a mortality rate up to 100% (3). During the mid 1980's a deletion mutant, namely the porcine respiratory coronavirus (PRCoV) with no or very limited pathogenicity emerged and spread around the world (4). The widespread PRCoV infection led to the gradual disappearance of TGE, due to the cross-protective immunity induced by the new virus. Recently more and more cases of piglet diarrhea with unclear aetiology are reported. This study is a summary of such cases. TGEV genome was detected with the parallel presence of PRCoV by polymerase chain reaction (PCR) in swine herds where pigs were showing signs of diarrhea.

Materials and methods

Samples (feces, lungs, small intestine and lymph nodes) were collected at 14 farms, among them 4 affected by diarrhea. RNA from homogenized samples was isolated by the Viral Gene-spin™ Viral DNA/RNA Extraction Kit (iNtRON Biotechnology) and after reverse transcription, tested for the presence of TGEV/PRCoV by polymerase chain reaction. The primers were TGE2: 5'-AAGGAAGGGTAAGTTGCTCA-3' (binding at 20282-20301 nt of the Purdue 115 strain) and TGE3: 5'-GGTCCATCAGTTACGCCGAA-3' (21538-21518 nt) flanking 1258 bases of the spike (S) gene, including the usual site of deletion for PRCoV. Size of the amplicons was tested by agarose gel electrophoresis and bands corresponding to TGEV-S and PRCoV-S were purified, sequenced and analysed.

Results

The farms, based on their TGEV/PRCoV profiles could be divided into three groups. In the first group pigs that suffered from clinical signs associated with enzootic TGE had either PRCoV- or TGEV-S gene sequences in their samples (550bp and 1250 bp respectively). In the second group of animals also with clinical signs of enzootic TGEV a variety of S gene fragment sizes were detected. Besides the full size fragment of the original TGEV-S smaller sized amplicons were also detected in the same animals, dominating among them one with an approximate size of 600 bp and another with an approximate size of 250 bp, as judged by the agarose gel electrophoresis. The third group consisted of animals that did not show clinical signs of TGEV infection. In these farms the TGEV-S genes were not detected, the S gene amplicons were of approximately 550-600 bp in length, consistent with what would be expected in PRCoV positive animals.

Discussion

Today TGEV is usually considered as a disease of the past. However, there has been occasional reports of TGEV seropositivity (1, 2), indicating that the virus was still present, but at levels below the threshold of clinical manifestation. The re-emergence of TGEV was confirmed in our study by sequencing the PCR generated amplicons targeting the spike gene. Apart from the deletion at the site of the S gene the sequences were identical in PRCoV and TGEV cases. The reason for the re-emergence of the pathogenic coronavirus is not fully understood. Based on the widespread presence of porcine circoviruses, also present in the examined herds, which are well known for their immunosuppressive nature, it was speculated and later confirmed by serological tests (not shown) that there is a decreased cross protection between PRCoV and TGEV. It is our opinion that this decrease in cross protection could be in the background of the TGEV induced clinical problems.

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CENSA FACING EMERGING AND RE-EMERGING VIRAL DISEASES OF SWINE. A TRANSMISSIBLE GASTROENTERITIS (TGE) CASE.

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Introduction

CENSA, Centro Nacional de Sanidad Agropecuaria, from Cuba, was founded in 1969 with the Mission to "Contribute to preserve and enhance animal, plant and human health" with the main objectives: To diagnose or discard exotic diseases in animal and plants. On animal branch, since the very beginning, CENSA has been involved in the two emergency of African Swine Fever (ASF) (1), appearing for the first time in the American Continent in 1971, Classical Swine Fever (CSF) (1993 (2) and 2002), Swine Transmissible Gastroenteritis (3) (TGE) (1993) until recently with the diagnosis of porcine parvovirus (4) (PVP), torqueteno sus virus (TTsusV) and porcine circovirus type 2 (PCV2) (5), in many other situations, CENSA has demonstrated its efficient capacities in human resource and infrastructure. Training researchers on this subject in centers of excellence and the constant information searched about the state of art in the world has been the main causes of this success.

In the particular case of transmissible gastroenteritis of swine (TGE) in 1993, the way of facing the diagnostic with the lack of reference tests contributed definitively to the success of its control program. This is an aspect characterizing CENSA as a National Reference Center for the diagnostic to the service of the Veterinary Medicine Institute (IMV), which is the authority body of animal health in Cuba

Materials and methods

During an emergency of a Syndrome in swine characterized by liquid diarrheas, high mortality rate in offspring, diarrhea and agalactia in their mother sows, diarrhea in other categories, without fever, the IMV immediately conformed a multidisciplinary and multi-institutional working group ad hoc preceded by the National Headquarter of the Civil Defense and under the direct attention of the Minister of Agriculture in functions and this situation was declared as national emergency.

CENSAs General Direction organized a multidisciplinary working group which integrally approached the diagnostic (Epidemiology, clinic, pathology, bacteriology, virology, toxicology, scientific-technical information, among others).

Results

From the clinic-epidemiological point of view it was evaluated as a lethal diarrheic syndrome in swine offspring with a recovering in older categories with an epidemic characteristics and signs of high transmissibility and a clear pattern of dissemination, the presence of bacterial or toxic agents was discharged and from the virological point of view the presence of a coronavirus in feces was confirmed by Electronic Microscopy and identified by RT-PCR (6) and sequencing.

Discussion

TGE was not included among the diseases constituting a threat for Cuba due to characteristics such as being a disease from template countries and with epidemic waves observed during the coolest months of the year For this reason Cuba and CENSA were not prepared for facing it from the diagnostic point of view. However, CENSAs attitude allows concluding the diagnostic, contributing decisively to introduce measures by the IMV for the contention of TGE to the originally affected province.

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VALIDATION OF A COMMERCIAL ELISA KIT FOR DETECTION OF ANTI-SWINE VESICULAR DISEASE VIRUS (SVDV) ANTIBODIES

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Introduction

Swine vesicular disease (SVD) is a contagious viral disease of pigs characterized by fever and vesicles in the mouth and on the snout, feet and teats. The illness may be subclinical, mild or severe, but rarely fatal. The importance of this disease stems from the fact that it cannot be clinically distinguished from foot-and-mouth disease (FMD). Outbreaks of SVD are therefore assumed to be FMD until laboratory tests prove otherwise.

Serology is used for SVD surveillance and export certification. While virus neutralization is the prescribed test for international trade, it is time-consuming, and 2-3 days are required to obtain results. In contrast, results may be obtained by ELISA in only a few hours.

ID VET has developed the ID Screen® Swine Vesicular Disease Competition ELISA to detect antibodies directed against the SVD virus in pig samples. The method used by this kit is described in the OIE Manual for Terrestrial Animals, Chapter 2.1.3. It is based on the monoclonal antibody 5b7 developed by Brocchi et al. This abstract reports validation data obtained for this ELISA. The main parameters evaluated are detectability, specificity, and sensitivity.

Materials and methods

Analytical sensitivity: Analytical sensitivity was evaluated using the reference sera produced by the OIE Reference Laboratory (Istituto Zooprofilattico Sperimentale, Brescia, Italy). The Secondary Reference Serum diluted 1:4000 corresponds to the Primary reference Serum RS4.

Specificity: 837 sera from non-infected regions (kindly provided by the Laboratoire Départemental 22 and Acseciate) were tested.

Sensitivity: 87 sera from naturally-infected animals were tested, including 35 sera from Italy and 52 sera from Spain. These sera were from infected herds and were classified as SVD-positive by the virus neutralization test (VNT).

Repeatability: Repeatability was evaluated by calculating the coefficient of variation (CV) for 96 repetitions of the secondary reference serum of IZS Brescia (equivalent to RS4), and 96 repetitions of the negative control of the kit.

Results

Analytical sensitivity: The secondary reference serum was detected diluted 1/4000. The experimental infection sera RS1-6 were detected as of 5 days post-infection.

Specificity: When doubtful results (n=7) are considered negative, the observed test specificity is 99.64% (CI95: 98.95%-99.88%).

Sensitivity: All sera were classified as positive by the ID Screen ELISA. The observed sensitivity was 100% (CI95: 95.8%-100%). The same sera were also tested by the OIE reference laboratory (IZS Brescia). Correlation between IDVET results and IZS Brescia results was 100%.

Repeatability: The CVs obtained were found to be between 3 and 8%.

Discussion

Specificity of the ID Screen® Swine Vesicular Disease Competition ELISA was 99.64% (CI95: 98.95%-99.88%) on the negative samples tested. Sensitivity was measured to be 100 % (CI95: 95.8%-100%). This ELISA is an easy-to-use, flexible and reliable test which gives results in two hours

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P.250

BIOCHEMICAL AND ANTIGENIC DIFFERENCES BETWEEN THE HEMAGGLUTININ-ESTERASE PROTEINS FROM TWO LINEAGES OF PORCINE TOROVIRUS

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Introduction

Toroviruses (ToV) are enveloped viruses with a positive single stranded RNA genome, that belong to the *Nidovirales* order. Four torovirus species have been described: equine torovirus (EqToV or BEV), bovine torovirus (BToV), porcine torovirus (PToV) and human torovirus (HToV). ToV are host-specific and cause enteric infections that can provoke severe diarrhea and dehydration (1).

The first two thirds of the ToV genome encode the non-structural proteins, whilst the structural proteins are encoded by four open reading frames (ORF) located at the 3´end. The nucleocapsid (N) protein interacts with the RNA molecule, and both are enclosed by the envelope membrane where the membrane (M) protein spans. The other structural proteins are the spike (S) and the hemagglutinin-esterase (HE) which are glycosilated transmembrane proteins whose hydrophilic domains protrude from the membrane (2). S is involved in cell receptor recognition. The ToV hemagglutinin esterase protein (HE) has been described as a receptor-destroying enzyme, since it holds a receptor binding domain and an acetyl-esterase catalytic region, but its function during the viral cycle is still unknown. In order to shed some light on HE functionality, two previously described PToV HE genes from two different genetic lineages (represented by P4 and Markelo strains) (3) have been biochemically and antigenically characterized in this study.

Materials and methods

In the course of an epidemiological survey about PToV in Spain, several viral strains were identified in field faecal samples (3). The HE genes from two PToV strains were identified in the same animal at different ages 52.7 and 52.11, and belonging to two PToV lineages were cloned. Recombinant vaccinia viruses (rVV) carrying these HE genes in the hemagglutinin (HA) locus of VV genome were produced. The correct expression of the HE proteins was confirmed by Western blot analysis with anti-HE specific antibody. As control, a rVV (rVV-HA-) only lacking the VV HA gene was generated similarly.The acetyl-esterase activity in the rVV- infected cell extracts was measured by pNPA assay (5) using an artificial substrate, as well as by receptor competition assays on mouse erythrocytes. The receptor binding capacity and specificity were analyzed by hemagglutination assays (HA) with red blood cells from different animal species. To investigate potential antigenical differences between these two HE proteins hemagglutination-inhibition tests were carried out with serum samples from piglets from the same farm where PToV had been detected.

Results

The two PToV HE proteins corresponding to lineages P4 and Markelo (3) were expressed as 65 kDa proteins in extracts from BSC40 cells infected with the corresponding rVV. These extracts showed acetyl-esterase activity when incubated with pNPA, while extracts form the control virus rVV-HA- did not show any activity. Moreover, incubation of mouse erythrocytes with extracts from HE expressing cells at 4ºC causes hemagglutination, but shifting the temperature to 37ºC cause activation of the acetyl-esterase function of HE proteins, and cessation of the hemagglutination reaction. Differences in the receptor binding capacity and specificity between these two HE proteins were determined by performing the hemagglutination assays with erythrocytes from different animal species Only the HE protein from PToV 52.7 showed the capacity to hemagglutinate chicken erythrocytes. Specificity differences were also determined by competition assays with mouse erythrocytes. On the other hand, hemagglutination-inhibition assays with serum samples collected from the same piglet from which the two PToV strains were isolated, and from its siblings clearly demonstrated that the proteins from the two HE lineages show different antigenic characteristics.

Discussion

In this study the receptor specificity of two naturally occurring PToV-HE proteins has been analyzed providing further insight into the infective process of this porcine emerging pathogen. The biochemical and antigenic characterization of both isolates, obtained from the same piglet at different time points (3), indicate a distinct substrate and enzymatic activity, defining a dynamic process of infection where the nature of receptor availability would favour the presence of a certain PToV isolate over others.

Acknowledgements

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EPIDEMIOLOGY PROJECT IN HAITI: RISK FACTORS RELATED WITH THE PRESENCE OF TESCHOVIRUS ENCEPHALOMYELITIS DISEASE

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Introduction

The Teschovirus encephalomyelitis virus has been described for the first time in the West Indies in 2009 in Haiti. The virus was identified as PTV-1 strain. It has been responsible for important direct animal losses and has significant economic impact on the familial farms (1). The disease is highly contagious and it has spread very fast within Haiti. This spurred the local veterinary services to study the factors influencing disease circulation in order better understand the epidemiology of this disease in Haiti and to prepare an adapted and efficient control protocol.

Materials and methods

Field investigations were carried out over a ten day period in the Haitian districts which declared the greatest number of PTV-1 suspicions in Artibonite and Bas-plateau regions (*Figure 1*).

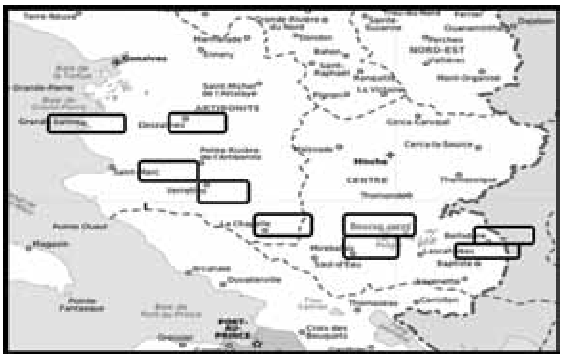

Figure 1: Study Area

A questionnaire was filled in on each farm to describe the animal management practices (housing, feeding, reproduction, animal movements…) and medical aspects (disease history, vaccination, treatments, sanitary visits).

In each farm, blood was taken from both clinically ill and apparently healthy pigs. If a pig showed PTV-1 clinical signs (hindquarter paralysis) with very reserved prognosis, it was euthanized to sample tissues, with financial compensation provided to its owner.

Serological and molecular diagnosis were performed by three laboratories (FADDL and Ames Iowa in US and CENSA in Cuba) to test for PTV-1 and the potential co-circulation of other swine pathogens (PRV, PRRS, EMC, H_1N_1, H_3N_2 viruses, CSF, PCV).

Data were included in a database and are being analyzed (case-control study, univariate analysis and logistic regression) by Haiti veterinary services and the coordination unit of the Caribbean Animal Health Network (CaribVET).

Results

109 blood samples and 63 tissue samples were collected in 111 pigs from 75 farms. Diagnostic results showed that 49.5 % of serum sample pigs were positive for antibodies to PTV-1. Serum was also found positive for antibodies to PRRS, CSF, PCV and influenza viruses (2).

The ongoing epidemiological and virological results analysis of data will provide identification of factors associated with seropositivity of farms and they will be object of future publication.

Discussion

The identification of risk factors associated with PTV circulation in farms in Haiti and the identification of other immuno-suppressive viruses co-circulating with PTV-1 virus will help with providing appropriate recommendations: best vaccination strategy, animal management and practices in the farm (farmers sensitization campaign). This work was performed as a training project in epidemiology for Haiti veterinary services within a Veterinary epidemiologist / paraepidemiologist project (VEP). This work was co-organized by USDA-APHIS, IICA and CIRAD Guadeloupe within the CaribVET network. The goal is that this project will eventually contribute to improved control of the disease and prevention of PTV spread to neighboring countries.

Acknowledgements

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ROUTINE COLLECTION OF EPIDEMIOLOGICAL INFECTIOUS DISEASE DATA IN PIG POPULATIONS USING ORAL FLUIDS

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Introduction

Research has shown that oral fluid specimens can be used to monitor the circulation of porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), and influenza A virus (IAV) in swine populations using polymerase chain reaction (PCR) assays (1-4). The purpose of the study described herein was to determine if on-going on-farm research based upon surveillance could be achieved through the routine collection and testing of oral fluid samples.

Materials and methods

Pigs in 10 commercial wean-to-finish barns on 10 different farms were monitored for the circulation of PCV2, PRRSV, IAV, and Torque teno virus genogroups 1 (TTV1) and 2 (TTV2) using oral fluid specimens. Oral fluid samples were collected from 6 pens at each site starting at the time of pig placement (~3 weeks of age) and continuing thereafter at 2-week intervals for a period of 18 weeks. The diagnostic results were analyzed for changes in the rate of detection in pens and barns over time.

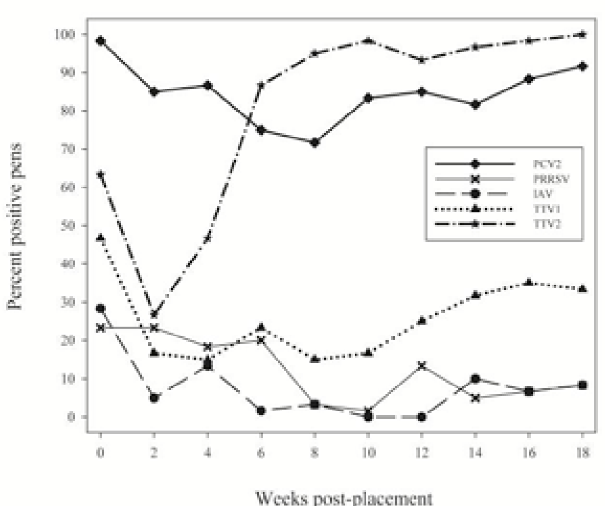
The presence of PCV2, PRRSV, IAV, TTV1, and TTV2and 2) in oral fluid samples was determined using PCR assays. A quantitative real-time reverse transcription (RT)-PCR assay was used to detect PRRSV or IAV RNA. Detection of PCV2 DNA, a real-time PCR was performed. TTV1 and TTV2 DNA were detected using a gel-based nested PCR.

Blood samples were collected from 5 randomly-selected pigs in each of the 60 monitored pens at the time of pig placement and during the week of the final oral fluid collection for a total of 600 serum samples.

Results

The PCR data on the 600 oral fluid samples are summarized in Figure 1. Overall, 508 (85%) were positive for PCV2, 73 (12%) for PRRSV, 46 (8%) for IAV, 483 (81%) for TTV2, and 155 (26%) TTV1. Fifteen (3%) of samples were negative for all 5 agents, 117 (20%) were positive for one agent, 289 (48%) for 2 agents, 149 (25%) for 3 agents, 27 (5%) for 4 agents, and 3 (0.5%) for all 5. The most common agent combinations were PCV2+TTV2 (239, 40%), PCV2+TTV1+TTV2 (88, 15%), and PCV2 alone (66, 11%). Cumulatively, one of these three combinations were detected in 393 (65%) of the 600 samples.

Figure 1. Percentage of pen oral samples testing positive via PCR.



Discussion

The ecology of infectious diseases and the impact of pathogens in pig populations require a method of collecting data that is repeatable and cost effective for serial sampling. Shedding of agents was of surprisingly long duration for PCV2, TTV1, and TTV2. PRRSV showed poor herd immunity, with some herds demonstrating long-term shedding and others showing re-infection. IAV showed surprisingly long shedding in some herds - even given known PCR false negatives based on serology (data not shown). Overall, the data suggested that the epidemiology of these agents described in text books needs to be re-written.

Acknowledgements

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TIME TRENDS OF IBERIAN WILD BOAR CONTACT WITH SELECTED INFECTIOUS AGENTS

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Introduction

Throughout Europe, Eurasian wild boar (*Sus scrofa*) populations have expanded both geographically and numerically in the last decades, driven by rural abandonment, agricultural production changes, and changes in game management among other factors. These free-living suids are known reservoirs for a number of viruses and bacteria that are transmissible to domestic animals and humans (1).

In Spain, wildlife disease surveillance has revealed wild boar as reservoirs for viruses such as Aujeszky's disease virus (ADV), porcine circovirus type 2 (PCV2) and hepatitis E virus (HEV) (2), and for bacteria including *Mycobacterium bovis* (3) and *Brucella suis* (4).

However, little is known regarding the temporal evolution of wild boar contact with these infectious agents. Recent developments in diagnosis and wildlife disease monitoring allowed addressing this issue. We hypothesized that wild boar from dense and more intensively managed populations, would show higher prevalences but more stable time trends as compared to wild boar from more natural regions within the Iberian Peninsula.

Materials and methods

Up to 4454 wild boar serum samples were obtained from hunter-harvested wild boar collected from 2000 to 2005 (Time 1) and from 2006 to 2010 (Time 2). We defined two study regions. The first one is Bio-Region 3 (BR3) as defined in (4). BR3 in south-central Spain is characterized by frequent fencing, feeding and watering. The second study region comprises the remaining parts of peninsular Spain, and is characterized by generally lower wild boar densities and limited artificial management.

Sera were stored at -20ºC and tested as shown in Table 1.

Infectious agent	Test used	Manufacturer or ref.
ADV	ELISA	IDEXX
PCV2	IPMA	(5)
HEV	ELISA	(6)
PRRS	ELISA	IDEXX
Brucella	ELISA	(4)
E. rhusiopatiae	ELISA	INGENASA
M. bovis	ELISA	(7)

Results

Table 2 shows the antibody prevalences observed for the 2 study regions and the 2 sampling periods.

	Bio-Region 3		Other BRs	
	Time 1	Time 2	Time 1	Time 2
ADV	55.8	54.8	29	34.4
PCV2	57.5	71*	51	41.3
HEV	28.6	21.5	16.7	20.3
PRRS	0	2.8	0	2.6
Brucella	46.9	42.9	31.8	24.4*
E. rhusiopat.	19.3	13.9	27.3	7.1*
M. bovis	36.2	34.5	3.1	7.6*

*= Chi^2 or Fisher's test significant at $p<0.05$.

Discussion

This survey evidenced significant differences between sampling periods for 4 of 7 infectious agents. Contact with ADV stayed stable, in marked contrast with recent declining trends among domestic pigs in Spain (http://rasve.mapa.es). PCV2 prevalence increased significantly in the intensively managed BR3 suggesting that risk factors such as spatial aggregation are still present (5). Contact with HEV stayed stable, in contrast to the increasing prevalences recorded among sympatric red deer (*Cervus elaphus*) from the same regions (8). Antibodies against porcine reproductive and respiratory syndrome virus (PRRS) were not detected in Time 1 but appeared in both study regions in Time 2. Regarding bacterial agents, contact with *Brucella* and *Erysipelothrix rhusiopathiae* declined outside of BR3 for unknown reasons, while contact with *M. bovis* increased out of BR3, possibly reflecting both an improved surveillance effort and a recent geographical expansion of the infection.

Acknowledgements

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THE USE OF SERUM PROFILES FOR CONTROL OF SWINE INFLUENZA VIRUS AND PORCINE CIRCOVIRUS TYPE 2 ON LARGE PIG FARM

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Introduction

Porcine circovirus type 2 (PCV2) infection has been associated with postweaning multisystemic wasting syndrome (PMWS), porcine dermatitis and nephropathy syndrome (PDNS), and porcine respiratory disease complex (PRDC). PCV2 is considered ubiquitous and domestic and feral swine appear to be the natural host (1).

Swine influenza (SI) is a common and important cause of respiratory disease in pigs. It is caused by type A influenza viruses which can act as a primary disease agents and as a part of PRDC (2).

PRDC is a multifactorial disease and a major economic problem for swine producers world-wide (3). Control of PRDC varies with each individual unit and system. Serology can gather information on which pathogens are circulating in a herd. Serum profile is testing groups of pigs at specified ages to detect when seroconversion to a pathogen is occuring in the unit. The most common method is to collect samples from several age groups on a single day. A less common but more accurate method is to follow individuals over time as they grow. The purpose of this study was to prepare serum profiles for SI virus and PCV2 for one large farm.

Materials and methods

38 serum samples from individual animals were tested on SI virus (SIV) and PCV2. Serum samples were taken at age of 2, 4, 6, 8, 11, 14, 17, 22 and 28 weeks. Pigs were raised in large Slovenian farm with 7000 breeding animals. For detection of specific antibodies following kits were used: against type A influenza viruses ELISA INGEZIM INFLUENZA PORCINA (Ingenasa) and against PCV2 ELISA INGEZIM CIRCO IgG (Ingenasa).

Results

The seroprevalence against SIV was between 81.5% and 100%. The seroprevalence was 100 % at 2 weeks of age, 94.7% at 4 weeks of age; the lowest was at 6 weeks of age (81.5%). The seroprevalence then increased to 100%, only at 17 weeks of age was a little lower (94.7%) (Figure 1). At 2 weeks of age seroprevalence against PCV2 from 84.2% decreased to 50% (at 6 weeks of age). At 8 weeks of age increased to 86.8% and at 14 weeks of age to 100% (Figure 2).

Figure 1. Serum profile of PCV2

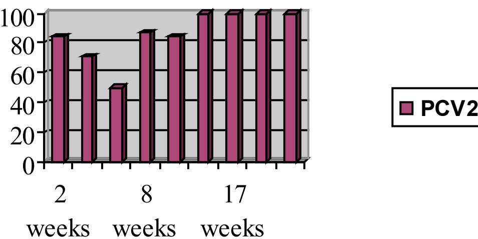
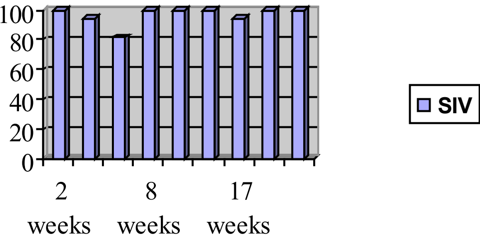


Figure 2. Serum profile of SIV



Discussion

Farm animals were not vaccinated against SIV and PCV2. Colostral antibodies against SIV and PCV2 persisted for about 4 weeks. The lowest seroprevalence against both viruses was detected in 6 weeks old pigs. The animals were probably infected with both viruses around 6 weeks of age. According to serum profiles vaccination of sows against SIV and PCV2 can be proposed for prolonged colostral immunity and better protection of animals against PMWS and SI.

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ECONOMIC IMPACT OF DISEASES ON PIG PRODUCTION WITH SPECIAL FOCUS ON EMERGING DISEASES

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Introduction

Infections cause substantial losses to the porcine production, but their economical impact is rarely discussed more than in general terms. The aim of this manuscript was to substantiate the impact of diseases in general and of some emerging diseases in particular.

Materials and methods

The revenues and expenses in Tab 1 were used to estimate losses and differences between herd categories. These figures were also used to estimate losses due to two emerging diseases; a) PMWS in 2004, the first year with the disease in Sweden (2), and b) PRRS which was introduced to and eradicated from Sweden in 2007 (1).

A base line for the national production was defined from herds affiliated to the PigWin control program in 2009. (Table 2). To visualise losses in conventional production the PigWin results were compared with the results of an SPF herd during the same year (3).

Table 1. Revenues and expenses per sow and year (€)

	Income	Holding/day	Death
Piglet		0	42
Weaner		0.35	46
Fattener	116	0.70	89
Sow		0.95	473

Results

The production is shown in Tab 2. The annual loss due to suboptimal production corresponded to 871 € per sow, which to 70% was caused by a retarded growth compared to the SPF herd, and to 20 % by preweaning mortality.

The mortality losses from weaning to slaughter was 67 € per sow in the Pigwin herds and 9 € in the SPF herd. Losses due to retarded growth was in mean 28 € per pig (616 € per sow), but ranged from 21 to 36 € per pig (top 25% and bottom 25% herds).

The mean net profit was 668 € per sow (slaughter income – rearing costs for the offspring). The net profit per sow in the top herds was 891 €, and 1,355 € in the SPF herd.

In the 16 herds deemed with PMWS in 2004 (2), the increased losses of 110 € per sow was dominated by mortality of growing pigs (Tab 3). Also the range in weight gain of growers increased, resulting in an estimated longer rearing time with 5 days to market weight. Reproduction was not affected.

The documented increased loss due to PRRS in 2007 corresponded to 224 € per sow until weaning. As PRRS was eradicated instantly (1), data post weaning do not exist. When estimated, the total losses increased to 443 € per sow and year (Tab 3).

Table 2. Mean productivity and net income per sow

	SPF	PigWin		
		Top 25%	Mean	Low 25%
Live born	29.7	29.2	27.0	22.9
Weaned	25.2	25.4	23.0	19.0
Slaughtered	25.1	24.5	22.0	18.1
Days * to SL	141	171	181	192
Net Income				
Slaughter, €	1355	891	668	428
Losses, €				
Mortality	199	226	244	244
Repro	6	8	11	15
Ret growth	0	515	616	646

Table 3. Annual losses per sow due to emerging diseases. (shadowed areas = estimated data)

	PMWS 2004		PRRS 2007	
	Δ	€	Δ	€
mortality				
Piglets	0	0	+17%	164
Weaners	+ 6%	63 €	+10%	105
Fatteners	+2%	39 €	+5%	98
Return/abort	0	0	+25%	60
Days * to SL	+5 days	8	+10 days	16
TOTAL doc		**102**		**224**
TOTAL est		**110**		**443**

Discussion

Despite that pigs in Sweden have a high health status and perform well, losses due to suboptimal production presumably related to infections were substantial. Preweaning mortality will never reach nil, but it is striking that the production would increase with 10% if one more pig could be weaned per litter

The losses were significantly increased in herds affected with PMWS and extensively in herds affected by PRRS - despite that the effect of these diseases were comparably mild compared to what has been described elsewhere.

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SEROLOGICAL DETECTION OF EMERGING VIRAL INFECTIONS IN WILD BOARS FROM DIFFERENT HUNTING REGIONS OF GERMANY

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Introduction

Emerging viral diseases like those initiated by Aujeszky's disease virus (PrV), classical swine fever virus (CSFV), PRRSV, swine influenza virus (SIV) and PCV-2 are still relevant in the swine population.

Although eleven EU member states are recognised to be free of Aujeszky's disease (AD) in domestic pigs, isolated cases of the disease are sometimes found in hunting dogs (1).

The wild boar population is also the reservoir for CSF. The last CSF outbreak in a swine herd in Germany was in North Rhine Westphalia in 2006.

Besides these OIE notifiable diseases, wild boars can be the source of other important viruses of domestic pigs, such as PRRSV, PCV-2, and SIV.

PRRSV infections in wild boars are diagnosed from time to time in Germany (2) and North Carolina (3). They might be a cause of infections in swine farms. PCV-2 infection is very common in the swine population and has caused enormous economic losses in the last decade. Its clinical significance is decreasing since the beginning of PCV-2 vaccination. In several studies, the occurrence of PCV-2 in wild boars was detected. PCV-2 virus and DNA were found in over 63% of examined wild boars in several regions of Germany (4). In North Carolina, 71% of the tested wild boars were PCV-2 antibody positive (3).

The same authors found antibodies against swine influenza in 91% of tested wild boars (3).

The aim of this study was to determine the seroprevalence of several emerging viral infections in wild boars in different hunting regions in Southern Germany.

Materials and methods

94 shot wild boars from 19 hunting regions in Bavaria, Rhineland-Palatinate and Baden-Wuerttemberg were examined. Sample collection took place from September to December 2010. Antibody titres to PrV, CSFV, PRRSV, SIV (H1N1) (all by IDEXX ELISA) and PCV-2 (IgM and IgG by Ingenasa ELISA) in blood serum were determined to see the prevalence of those infections in wild boars of the sampled regions.

Results

The results are summarised in Table 1. In one wild boar (over two years old) both PrV and PCV-2 IgG antibodies were detected. The other animals showed positive antibody results to at most a single viral infection. The four PrV antibody positive animals, as well as the two SIV antibody positive wild boars, came from two different hunting regions. The single PRRSV antibody positive result was just at the cut off level. All three PCV-2 IgM antibody positive animals were also IgG positive, and young (7, 20 and 35 kg body weight).

Table 1: Antibodies to several emerging viral infections detected in blood serum of 94 wild boars from Southern Germany

Antibodies to	No. positive	% positive
PrV	4	4,2
CSFV	0	0
PRRSV	1	1,1
PCV-2 IgG	15	16
PCV-2 IgM	3	3,2
SIV (H1N1)	2	2,1

Discussion

The hunting regions where the samples were taken are sparsely populated and contain large amounts of wild boars.

The results show that PrV antibodies are present in wild boars in Southern Germany, although this was not a very frequent finding. Still, a potential danger for hunting dogs and conventional swine farms must be considered.

CSFV antibodies were not at all found in this region.

PRRSV antibodies were also hardly found in the sampled wild boars.

Compared to the literature (3, 4), much lower numbers of PCV-2 seropositive wild boars were found in our study, Like in conventional pigs, acute PCV-2 infection in wild boars mainly affects young animals. This is confirmed with the simultaneous appearance of the early IgM and the IgG antibodies.

Only two SIV (H1N1) seropositive animals were found. Although swine influenza is very common in conventional pigs, it may be less common in wild boars.

In summary, the antibody prevalence to emerging viral diseases was low in this study. Nevertheless, the wild boar population may still be a source of infection for conventional pigs. This is especially important in the case of notifiable diseases, like AD.

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P.257
IBERO-AMERICA SWINE PRODUCTION NETWORK (IBEROSWINE)

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Introduction

Pork meat and its sub-products are very important part of the people's diet of many countries around the World (1). In Latin America, pork meat in addition to be an important source of protein, it also offers a prominent financial support for the small or family-run swine production. However, production practices and health status of the pigs in Latin American countries are broadly diverse and in some cases disadvantageous. In order to improve the swine production and heath status in those areas, in 2010, the Ibero-American Swine Production Network (IberoSwine) (2,3) was created. IberoSwine is supported by the Latin America Science & Technology Development Program (CYTED).

Materials and methods

IberoSwine are integrated by swine producers, veterinarians, animal researchers and people from the veterinary industry from more than 14 countries of Ibero-America. The people that integrate IberoSwine belong to the most important Universities and/or Research Centers from Ibero-America.

Swine health in Ibero-America represents an enormous challenge for all the people involved in swine production in these areas. For that reason, IberoSwine encloses professionals working in diverse health issues affecting porcine production, for instance, porcine influenza, a disease that affect the whole swine production in Ibero-America. There are other diseases of relevance to few countries, such as the porcine reproductive and respiratory syndrome (PRRS), which is a very important health concern in Mexico and Spain, compared to Brazil which bears a negative status or Chile the only Latin American country that has eradicated the virus. Other example includes the porcine circovirus, affecting most of the countries in Ibero-America, but its impact in swine production is very diverse. Some countries in Central America, the Caribbean, as well as South America are affected by the classical swine fever virus which represents an important economical concern for these countries. These diseases are just some of the health issues to which countries in Ibero-America faces everyday in swine production practices. It is of vital importance to confront them in order to improve the swine production. This challenge involves translating the knowledge generated in research labs to the people working in the field.

Results

At this moment, people involved in IberoSwine are interested in improving the health status of swine production in Ibero-America, with special emphasis in those caused by aforementioned viruses with the ultimately goal of eradicating the diseases and therefore improving the economy of many people involved in this activity. In addition, IberoSwine promotes the exchange of information, protocols, seminars, actualization courses and manuals. As a matter of fact, handbooks on porcine health management and disease control are being produced coordinately among participating countries. Also, scientific meetings were held in several places of Ibero-America, the last one in Costa Rica (2010) with special emphasis in PRRS, PCV2 and swine influenza. On the other hand, IberoSwine promotes the cooperation among partners for developing research and cooperation programs directed to relevant diseases of swine appearing or re-appearing in IberoAmerica. These are just some of the means that IberoSwine uses to improve swine production (2,3).

Discussion

The final aim of this network is the construction of collaborative projects among the members on this network to control pig diseases affecting swine production in Ibero-America and establishing common standards for the diagnosis and control of particularly important conditions such as PRRS, post-weaning multisystemic wasting syndrome, influenza or classical swine fever.

Acknowledgements

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