5TH INTERNATIONAL SYMPOSIUM ON EMERGING AND RE-EMERGING PIG DISEASES

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RESEARCH • PERFORMANCE • INTEGRITY
Dear Friends and Colleagues,

It is a great pleasure for Intervet to present you with this booklet which contains the proceedings of the Intervet Satellite Seminar, together with abstracts of those papers to be presented at the 5th International Symposium on Emerging and Re-Emerging Pig Diseases which are related to Intervet products, or which report on Intervet’s collaborative studies with various research institutes on topics of mutual interest.

The Seminar begins with Professor Joaquim Segales from the Veterinary School of the Universitat Autònoma de Barcelona, Spain, who will present data on a vaccination and challenge experiment involving four different PCV-2 isolates originating from different parts of the world. These PCV-2 isolates belong to different genetic clusters, providing an answer to the question of vaccinal protection against different field strains. As well as the data, Professor Segales will discuss the importance of keeping PCV-2 virus at a low level amongst piglets.

Professor Paolo Martelli from the University of Parma, Italy, will be discussing a trial designed to reveal the importance of European PRRS field virus in respiratory disease in pigs. While in the USA, the economic damage caused by PRRS virus-related respiratory problems is widely acknowledged and well documented, it has not been as clear, so far, under European conditions. With his unique trial design, Professor Martelli will present data to demonstrate that European field virus of PRRS can cause respiratory problems, and that even with a heterologous PRRS field virus challenge, a significant level of protection can be expected from Porcilis PRRS, the mlv vaccine based on a European strain of PRRS virus.

The seminar will close with a presentation by Dr Stefano Gozio on the flexibility of the use of Porcilis PRRS with other Intervet vaccines. There is ongoing pressure to reduce the number of injections given to animals, but the need for multiple vaccinations goes on increasing. Different farms need protection against different diseases, but combination vaccines cannot contain the mix of antigens appropriate for every farm, and the timing of administration can be different for different locations. What the market really needs can be summarized in the one word “flexibility”.

Intervet’s vaccines are based on the proprietary, patented, Diluvac Forte®; and the various antigens have been shown to be highly efficacious in this well-tolerated adjuvant. This brings a considerable benefit in that it allows for different Intervet products to be used together. Dr Gozio’s data will help veterinarians to make informed decisions when it comes to vaccine selection by taking advantage of this flexibility.

Wishing you an instructive and fruitful symposium,

Drs Alex A.S. Eggen DVM
Head Technical Service Swine Products
Intervet
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of the 5th International Symposium

on Emerging and Re-Emerging Pig Diseases
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Speaker: Joaquim Segalés
Title: Porcine circovirus type 2 (PCV2): strains, pathogenicity and vaccines

- DVM at the Universitat Autònoma de Barcelona (UAB) (1991)
- PhD in veterinary sciences, UAB (1996)
- Diplomate European College of Veterinary Pathologists (ECVP) (2000)
- Diplomate European College of Porcine Health and Management (ECPHM) (2004). Founding member.
- Current position: Associate Professor at the Pathology Department of the Veterinary School at UAB, and researcher at the Centre de Recerca en Sanitat Animal (CReSA)
- Research interests in swine diseases, and has been participating in research of pig infectious agents since 1993. Main areas: porcine reproductive and respiratory syndrome virus, Aujeszky’s disease virus, postweaning multisystemic wasting syndrome and porcine circovirus type2, Haemophilus parasuis, Mycoplasma hyopneumoniae, Hepatitis E virus
- Diagnostic activity on swine diseases at the Veterinary Pathology Diagnostic Service at the UAB since 1996
Introduction

Porcine circovirus type 2 (PCV2) is nowadays considered the essential infectious agent of a number of diseases collectively named as porcine circovirus diseases (PCVDs) (Segalés et al., 2005a). The role of PCV2 in some of these PCVDs is debatable but, from a pathogenic point of view, little doubts do exist to consider PCV2 as the needed cause for PMWS and PCV2-associated reproductive disease.

PMWS is considered, by far, the most significant PCVD from an economical point of view. Specifically, in 2004, it has been estimated that PMWS cost around 600 million Euros per year to the European Union (Armstrong and Bishop, 2004). It is very likely that similar levels of losses have occurred in the last two years in North America. Direct losses come from mortality in the nursery and fattening pigs, and from unthrifty pigs, unable to reach market weight. Indirect losses come from increased use of antibiotics in an attempt to control concurrent bacterial infections, and changes in farm management practices in an attempt to reduce the impact of PMWS.

The prevalence and severity of PMWS outbreaks have, to some degree, diminished during last years in some European countries as well as in South-east Asia. However, the disease is still significant or very significant in some countries of those geographical areas (Wellenberg and Segalés, 2006). On the other hand, PMWS has re-emerged importantly during last two years in North and South-America, being considered the number one disease in many countries (Carman et al., 2006; Cheung et al., 2007).

The re-emergence of PMWS in several parts of the world (mainly North-America) caused even more significant research efforts from both practical and scientific points of view. The former aspect has been mainly represented by the advent of PCV2 vaccination. After more than two years of sow vaccination in some European countries and more than one with both sow and piglet vaccination in North-America (Harding, 2007; De Grau, 2007; Francisco, 2007; Desrosiers, 2007; Segalés, 2007), it is clear that PCV2 vaccination seems to be one of the most successful vaccines applied in pigs ever. However, besides its excellent efficacy, little is known about the mechanism by which those vaccines work. On the other hand, several scientific aspects have been tackled during last five years, although many answers are still missing. One of these topics is the possibility that differences in pathogenicity among PCV2 strains do exist. If this would be the case, appropriate global epidemiological studies might help elucidating why PMWS started as an almost worldwide epizootic by 1995-97 while PCV2 strains have been circulating in the pig population at least several decades before the description of the disease.

Therefore, the objective of this presentation is to review some of the most up-to-date practical and scientific aspects of PMWS, and to describe an experimental trial on vaccinated pigs subsequently challenged with different PCV2 strains.
PMWS versus subclinical infection, PCV2 pathogenicity and PCV2 vaccination

One of the most difficult issues to explain is how PCV2 can cause disease in a proportion of infected pigs taken into account that it is a ubiquitous virus. The pathogenic mechanism has not been fully elucidated yet, but it is clear that different factors (usually referred as triggering or risk factors) seem to be related with a major or minor prevalence of PMWS in a given farm (Segalés et al., 2005a). On the other hand, differences between PMWS affected and PCV2-subclinically infected pigs have been defined from pathological, virological and immunological points of view (Figure 1).

Figure 2: Outline of the pathogenesis of PCV2 infection with particular emphasis on the potential outcomes of this infection.

From a practical point of view, PMWS affected pigs can be differentiated from PCV2-subclinically infected pigs based on the amount of PCV2 present in tissues and serum (Segalés et al., 2005b): definitively, PMWS affected animals harbour a significant higher amount of virus and also are able to shed higher amount of PCV2 (Figure 2). Therefore, it is not a surprise that the individual case definition for PMWS implies not only the existence of moderate to severe lesions in the lymphoid
tissues (lymphocyte depletion with granulomatous inflammation), but also moderate to high amounts of virus in tissues and serum (Segalés, 2002). Importantly, besides to ameliorate the effect of potential triggering factors, it seems that one specific way to control PMWS would be to down-regulate PCV2 replication; in other words, if we are able to impede PCV2 replication to reach certain threshold, we will probably control PMWS. One of the potential manners to achieve this objective is, obviously, through vaccination strategies. As for all viral infections, the knowledge on innate and acquired immune responses to PCV2 should be compulsory to design an appropriate vaccine product. However, this knowledge is usually behind the development of vaccines and even the commercialisation and use of those vaccines under field conditions. Based on the current scientific data on PCV2 infection, it would apparently be necessary that such vaccines elicit significant humoral neutralising as well as cellular immune responses.

Some vaccine studies already existed before the commercialization of PCV2 vaccines. PCV2 vaccine prototypes described in the literature include experimental products based on inactivated PCV2 isolates (Pogranichny et al., 2004), a chimeric virus (PCV2 capsid gene cloned in the backbone of the non-pathogenic porcine circovirus type 1 genome) (Fenaux et al., 2004), and DNA and sub-unit vaccines (Blanchard et al., 2003). In all cases, and using experimental approaches in which PMWS was rarely reproduced, a significant decrease of lesional severity in lymphoid tissues and shorter PCV2 shedding and viremia length were achieved. These prototypes were never tested under field conditions and its real efficacy was really unknown, but this situation changed in 2004. In that year, an inactivated, adjuvanted PCV2 vaccine for use in sows and gilts was commercialised and in use under special license in some European countries (France and Germany) (Charreyre et al., 2005). The same vaccine was available in Denmark and Canada since 2006 and, in a more restrictive situation, also in other European countries. Nowadays, more than a half million of sows has been vaccinated in Europe and a similar amount in Canada, obtaining significant reductions in mortality and percentage of runts in the postweaning area. More recently, in 2006, three new PCV2 vaccines for use in piglets came to the market in North-America (Canada and/or USA). One of them was based in the inactivated chimeric virus cited above while the other ones were based on a PCV2 protein expressed in a baculovirus system. Most of the reports in regards the use of those vaccines have also shown a significant improvement of postweaning (nursery and fattening) mortality, being in some cases extremely positive.

Therefore, PCV2 vaccines seem to be one of the major weapons that can be used to control or prevent PMWS in those severely affected farms (De Grau, 2007; Francisco, 2007; Desrosiers, 2007). Surprisingly, in some farms, the overall mortality levels achieved after PCV2 vaccination in the postweaning area dropped to values even lower than those previous to the PMWS outbreak. These results may suggest that those vaccines, at least in some cases, may have been also able to counteract certain unknown or
subclinical effects caused by or associated to PCV2 infection. This point will need further confirmation as well as the appropriate scientific studies on the real effect of PCV2 “subclinical” infections.

Moreover, the clear efficacy of PCV2 vaccines in controlling PMWS further supports the central role of PCV2 in PMWS causality. However, this situation does not change the status of PMWS as a multifactorial disease; therefore, other concomitant factors may have a worsening effect in PCV2 infected piglets and the improvement of management, control of concurrent diseases, immunostimulation, etc. must still be considered when designing a plan to prevent PMWS in a given farm.

Another important point potentially related with the efficacy of PCV2 vaccines came from Canada in 2005 (Carman et al., 2006). An epidemiological study indicated a higher frequency of a certain restriction fragment length polymorphism (RFLP) pattern of PCV2 strains (pattern 321) more frequently associated to PMWS cases than other RFLP patterns (patterns 422 and others). Therefore, this was the first epidemiological study that pointed out clearly the possibility that different strains of PCV2 may vary in their pathogenicity. If this would be the case, then, a question that automatically comes up is the fact whether available vaccines are able to counteract all PCV2 isolates or just some of them.

However, PCV2 pathogenicity variability is not an easy and clear-cut issue. In an experimental study using two PCV2 strains (Opriessnig et al., 2006), one isolated from a PMWS case (“high virulent strain”) and another from a healthy pig (“low virulent isolate”) showed different lesional degrees in lymphoid tissues. However, in spite of these differences among groups (supporting the hypothesis of different pathogenicity among PCV2 strains), no clinical disease was observed in any of the experimentally infected pigs from both groups. Moreover, the theoretical predicted RFLP pattern of these two isolates was 422-like. On the other hand, other molecular epidemiological studies performed in France (De Boissesson et al., 2004) and The Netherlands (Grierson et al., 2004) have shown that there is no apparent virulence marker in the genome of PCV2 isolates coming from PMWS affected pigs compared to those coming from healthy pigs. In fact, when using the predicted theoretical RFLP patterns, almost all PCV2 isolates from these studies were 321, independently of coming from PMWS affected or healthy pig. Finally, a PCV2 isolate from a healthy pig from a healthy farm from a country that was “free” of PMWS at that time (1993 in Sweden) was able to cause PMWS in a model with porcine parvovirus co-infection (Allan et al., 2003). Surprisingly or not, that PCV2 isolate corresponded to RFLP pattern 22.

Definitively, the potential variation among PCV2 isolate pathogenicity is still an open debate and surely will continue being studied in the next few years. However, and although certain differences have already been described, it also seems that whatever PCV2 isolate within the adequate environmental conditions (let’s read concomitant presence of triggering factors at the “right” moment) would be potentially capable of producing PMWS. If this variable pathogenicity among PCV2 isolates is a likely explanation in regards the sudden increase of PMWS prevalence by late 90s in Europe and Asia or in 2005 in North-America, remains to be elucidated.

**Porcilis PCV: strain differences and vaccination**

Based on the apparent fact that PCV2 strain pathogenicity variation may occur, it is logical to wonder about the efficacy of vaccines against different strains. In fact, phylogenetic analyses clearly indicate that at least two differentiated groups of PCV2 sequences do exist. It is surprising, however, to see that different nomenclatures are concurrently being used to name those different groups of PCV2 strains (Table 1), and a unified criterion for definitive designation is needed.
Therefore, we designed an experiment to test the efficacy of a PCV2 vaccine in a challenge model using four different PCV2 isolates, two corresponding to clade or group 1 and two to clade or group 2. The used vaccine was Porcilis PCV (Intervet), a subunit vaccine containing a PCV2 protein expressed in a baculovirus system.

For that experiment, 72 3-week-old, colostrum-fed piglets were allocated in the experimental facilities of CReSA (Centre de Recerca en Sanitat Animal, Spain). Experimental design is summarised in Table 2. The use of colostrum-fed pigs was intended to reproduce natural field conditions.

Different parameters were considered in the evaluation of the vaccine study:

- Clinical signs (daily)
- Body weight (days 0, 14, 29, 35, 42 and 49 of experiment)
- Rectal temperatures (from days 29 to 49 of experiment, every two days)
- Viremia (measured by quantitative PCR in serum) (days 0, 14, 29, 35, 42 and 49 of experiment)
- Shedding (measured by quantitative PCR in nasal cavity and faeces) (days 0, 14, 29, 35, 42 and 49 of experiment)
- Histopathology and presence of PCV2 in tissues by in situ hybridization (ISH)
- Total PCV2 antibodies (measured by immunoperoxidase monolayer assay, IPMA) (days 0, 14, 29, 35, 42 and 49 of experiment)
- PCV2 neutralising antibodies (measure by virus neutralisation test, VNT) (days 0, 29 and 49 of experiment)

Table 2. Experimental design.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Number of pigs</th>
<th>Days of experiment*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>A</td>
<td>7</td>
<td>V</td>
</tr>
<tr>
<td>B</td>
<td>7</td>
<td>V</td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>V</td>
</tr>
<tr>
<td>D</td>
<td>7</td>
<td>V</td>
</tr>
<tr>
<td>E</td>
<td>7</td>
<td>V</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

*V = Vaccination; N = necropsy; C = challenge (C1 = PCV2 of clade 1; C2 = PCV2 of clade 2; C3 = PCV2 of clade 2, C4 = PCV2 of clade 1)

Vaccinations were done by intramuscular route at the start of the experiment (right side of the neck) and 2 weeks later (left side of the neck) in 35 pigs (7 en each treatment group). Two ml of Porcilis PCV were applied per animal in each vaccination. The rest of 37 pigs received the same amount of PBS at same days. On day 29 of experiment, all pigs from all treatment groups but E, were inoculated with 2x10^4.2 TCID₅₀/pig of the corresponding virus.
No PMWS was generated in any of the PCV2 infected pigs (groups A to D). Minimal histopathological
PMWS-like lesions were detected in 7 pigs, all of them corresponding to non-vaccinated animals
included in groups A, B and D. No histopathological lesions were detected in most of the pigs (none
in groups C and E).

Serological and virological results indicated that most of the animals from those groups got a
subclinical PCV2 infection. No positive quantitative PCR results were obtained on days 0, 14 and
29 of experiment in any of the pig groups; results of viremia and shedding during the rest of the
experiment are summarised in Table 3. Significant differences in viral load and/or proportion of
positive animals were detected between vaccinated and non-vaccinated groups at different time points
in serum, nasal cavity and faeces.

From a serological point of view, and in spite of the presence of maternal humoral immunity at
the starting of the experiment, all vaccinated pigs seroconverted with significant higher PCV2
titres compared to those of non-vaccinated pigs at 29 days of experiment. Those differences were
maintained at days 35, 42 and 49 of experiment. Similar results were obtained with the VNT. All
vaccinated pigs developed a neutralising antibody response, which was significantly higher than that
of non-vaccinated ones at days 29 and 49 of experiment.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Number of pigs</th>
<th>Actions performed*</th>
<th>Number of positive pigs by quantitative PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 35</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S N F</td>
</tr>
<tr>
<td>A</td>
<td>7</td>
<td>V-C1</td>
<td>0 7 2</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>C1</td>
<td>1 7 6</td>
</tr>
<tr>
<td>B</td>
<td>7</td>
<td>V-C2</td>
<td>0 3 1</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>C2</td>
<td>0 7 0</td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>V-C3</td>
<td>0 7 0</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>C3</td>
<td>0 7 0</td>
</tr>
<tr>
<td>D</td>
<td>7</td>
<td>V-C4</td>
<td>0 5 0</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>C4</td>
<td>0 7 0</td>
</tr>
<tr>
<td>E</td>
<td>7</td>
<td>V</td>
<td>0 0 0</td>
</tr>
</tbody>
</table>

*V = vaccinated; C = challenged (C1 = PCV2 of clade 1; C2 = PCV2 of clade 2;
C3 = PCV2 of clade 2, C4 = PCV2 of clade 1)

Therefore, under the conditions of the present study, it can be concluded that Porcilis PCV was able to
prevent viremia against the challenge with 4 different PCV2 strains, as well as to overall decrease the
PCV2 load at nasal cavity and faeces. Moreover, Porcilis PCV was able to elicit high titres of antibodies
to PCV2, including neutralising ones.

Acknowledgements

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for technical assistance.
References


Segalés J (2007). Porcine circovirus disease (PCV2) – have we won the war in Europe? Advances in Pork Production 18: 49-56.

Intervet Satellite Symposium

Speaker: Paolo Martelli
Title: The importance of PRRS virus in respiratory disease in pigs

• Full professor of Internal Medicine – University of Parma (Italy)
• Head of the Department of Animal Health – University of Parma (2003 – )
• Member of Evaluation Board of the University of Parma (2005 – )
• “Physical Examination” and “Internal Medicine - Clinics of Large Animals” at the Faculty of Veterinary Medicine – Faculty of Veterinary Medicine (University of Parma)
• “Pig Medicine” at the post-graduate School of Specialisation in Pig Medicine at the University of Parma
• “Large Animals Medicine” at the post-graduate School of Specialisation in Animal Health and Husbandry at the University of Parma
• “Pig Medicine” at the post-graduate School of Specialisation in Pig Medicine at the University of Turin
• More than 200 publications
• 2 original text books on Pig Diseases and vaccinations (in Italian) translation into Italian of “Diseases of Swine”
• Founding Father and Diplomate of the European College of Porcine Health and Management
• Chief Editor of O&DV (Italian Journal of Veterinary Medicine) (1998 – )
• Members of the Italian Pig Veterinary Society
• Members of the Italian Veterinary Science Society
• Members of the American Association of Swine Veterinarians
• Member of Scientific Committees of Congresses and Symposia
THE IMPORTANCE OF PRRS VIRUS IN RESPIRATORY DISEASE IN PIGS

Paolo Martelli
DVM, Diplomate ECPHM
Full Professor of Internal Medicine
Department of Animal Health, University of Parma - Italy

Background

- 88% of the total cost of PRRSV infection in USA is attributable to increased mortality rates and decreased growth performances in nursery and grower-finisher phases.

- Genetic divergence and presumably at least some antigenic diversity among PRRSV isolates raise questions about immunity and particularly cross-protection.

- Although it has been reported that vaccination with MLV provides incomplete heterologous protection against PRRSV infection, multiple experiments have revealed substantial reduction in viremia, reduction in lesions and clinical signs following heterologous challenge.
Vaccine efficacy in laboratory conditions

- Respiratory tract protection upon challenge of pigs vaccinated with attenuated PRRS virus vaccines (Labarque et al. Vet Microbiol 2003)
- Impact of genetic diversity of European-type PRRSV strains on vaccine efficacy (Labarque et al. Vaccine, 2004)
- Different EU vaccines against PRRSv have different immunological properties and confer different protection to pigs (Diaz et al Virology 2006)
- Reproductive performances of gilts following vaccination and subsequent heterologous challenge with European strains of PRRSV (Scortti et al. Theriogenology, 2006)
- Protection and immune response in pigs intradermally vaccinated against PRRS and subsequently exposed to a heterologous EU (Italian Cluster) field strain (Martelli et al. Vaccine, 2007)
- Assessment of the efficacy of commercial PRRS vaccines based on measurement of serologic response, frequency of gamma-IF-producing cells and virological parameters of protection upon challenge (Zuckerman et al., Vet. Mic, 2007)

Vaccine efficacy in laboratory conditions

In laboratory conditions
- presumptive evidence for vaccine-induced protective immunity against the heterologous challenge strain are provided by finding that viremia following challenge is generally less (incidence) and significantly less (titers) in vaccinated pigs than in nonvaccinated pigs.
- The absence of virulent-virus-induced clinical signs and macroscopic lesions in nonvaccinated as well as in vaccinated pigs precludes a more definitive evaluation of the magnitude of protective immunity provided by vaccination.
Effects of ML PRRSV vaccine in challenge trials

- Homologous strains
  - Complete protection against viremia and disease
  - Positive effect on virus persistence
  - Reduction in the level of viral load in the tissues

- Heterologous strains
  - Infection is not prevented
  - Reduction of duration and magnitude of viremia
  - Reduction of clinical signs
  - Viral loads in positive tissues are not reduced
  - Repeated vaccination (MLV) reduces viral shedding
  - In sows: statistically significant level of protection in regards to the incidence of congenital infection, reproductive performance, and pig health and viability.

Vaccine efficacy in field (on farm) conditions

- PRRSV-EU vaccine protects pigs against infection with Italian-like field strain (Stadejek et al., 2005)

- Impact of a modified-live porcine reproductive and respiratory syndrome virus vaccine intervention on a population of pigs infected with a heterologous isolate (Cano et al., vaccine, 2007)

- Effect of vaccination with a modified-live porcine reproductive and respiratory syndrome virus vaccine on dynamics of homologous viral infection in pigs (Cano et al., AJVR, 2007)

- Efficacy of a ML PRRSV vaccine to control respiratory disease in pigs naturally exposed to a virulent heterologous field virus (Martelli et al. to be published)
Efficacy of ML EU-PRRSV vaccine (DV strain) to control respiratory disease in pigs naturally exposed to a virulent heterologous field virus.

Clinical and virological findings, measurement of serologic response and cell mediated immunity

(Martelli et al. Submitted for publication)

The aim of the study

- The research was inspired by the apparent paradox that European strains of PRRSV cause minimal respiratory signs under laboratory experimental conditions.

- The aim of this study was to evaluate the efficacy of vaccination (EU - ML PRRSV VACCINE) in pigs naturally exposed (on farm conditions) to a virulent heterologous field virus (Italian cluster).
Study design

Study design: source and housing of pigs (site 1)

- 30 conventional piglets
- PRRSV and M hyo free
- Weaned at 28 d → isolation barns
- Reared in isolation
Study design: housing during the nursery phase (site 2)

- **PRRS IM**
  - 35 days of age
- **PRRS ID**
  - 35 days of age
- **Controls**
  - un vaccinated

Study design: housing during the grower phase (site 3)

- **PRRSV field strain**: 84.7% homology (ORF 5) to the vaccine strain

At 60 d: the animals were moved to a recipient conventional 3rd site herd (grower-finisher production phase) with a history of respiratory disease (PRRS + M. hyo positive)

In the same barn with hundreds of other pigs

The percentage divergence in the ORF5 nucleotide sequence between the resident PRRS isolate and the DV strain (ML EU-PRRSV vaccine) was 15.3%
Study design: Clinical monitoring

- Pigs were monitored and scored daily for general and respiratory clinical signs throughout the post-exposure period (0 to 34 days).

- General Clinical Score (GCS)
  - Body (rectal) temperature
    - 0: T ≤39.5°C;
    - 1: T between >40.0 and ≤40.9°C;
    - 2: T ≥41.0°C
  - Appetite (0=normal or 1=anorexic);
  - Level of consciousness (0=normal or 1=compromised).

- Respiratory Clinical Score (RCS)
  - Respiratory disease score ranging from 0 to 4
    - 0=normal,
    - 1=tachypnoea when stressed,
    - 2=tachypnoea at rest,
    - 3=tachypnoea and dyspnoea at rest,
    - 4=severe tachypnoea and dyspnoea with laboured, jerky breathing
  - Coughing score (0=absent or 1=present);
  - Nasal discharge score (0=absent or 1=present).

- Overall Clinical Score was calculated by adding the general clinical score (GCS) and the respiratory clinical score (RCS).

Study design: blood samples

Blood samples
- VIROLOGY
  - PCR
    - Virus isolation and titration
- SEROLOGY
  - ELISA (S/P ratio)
  - IFA TEST
- Flow Cytometry
- IFN-γ
Results

Clinical monitoring

Mortality

<table>
<thead>
<tr>
<th>AGE</th>
<th>INCLUSION DAY 35 DAYS</th>
<th>EXPOSURE DAY 80 DAYS</th>
<th>END OF THE OBSERVATION PERIOD 114 DAYS</th>
<th>+60 DAYS LATER 174 DAYS</th>
</tr>
</thead>
<tbody>
<tr>
<td>IM</td>
<td>10/10</td>
<td>10/10</td>
<td>9/10</td>
<td>9/10</td>
</tr>
<tr>
<td>ID</td>
<td>10/10</td>
<td>10/10</td>
<td>10/10</td>
<td>10/10</td>
</tr>
<tr>
<td>C</td>
<td>10/10</td>
<td>10/10</td>
<td>8/10</td>
<td>6/10</td>
</tr>
</tbody>
</table>

Severe pneumonia: isolation of *Pasteurella multocida*
No detection of M hyo by PCR
Body (rectal) temperature

Temporal trend for var=fever01

GCS - Percent of animals with score > 0

RCS - Percent of animals with score > 0
GCS = General Clinical Score
(sum of fever + appetite + level of consciousness)

GCS (mean values) vs time

Cumulative GCS (mean values std to 100) vs time
RCS = Respiratory Clinical Score
(sum of MRS + cough + nasal discharge)

RCS (mean values) vs time

Cumulative RCS (mean values std to 100) vs time
Overall score: RCS+GCS

Overall score (mean values) vs time

Cumulative overall score (mean values std to 100) vs time
## Odds ratio - Summarizing table

Clinical features: comparison between groups (OR values and 95% c.i.). Only values for significant comparisons (chi-square, P<0.05) are reported.

<table>
<thead>
<tr>
<th></th>
<th>Body temperature (fever)</th>
<th>Nasal discharge</th>
<th>dyspnoea</th>
<th>appet01</th>
<th>resp01</th>
<th>cough01</th>
<th>consec01</th>
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</thead>
<tbody>
<tr>
<td><strong>IM vs ID</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>IM vs C</td>
<td>0.27; 0.20; 0.38</td>
<td>0.09; 0.31; 0.38</td>
<td>0.27; 0.17; 0.41</td>
<td>0.21; 0.14; 0.31</td>
<td>0.15; 0.11; 0.22</td>
<td>0.27; 0.19; 0.38</td>
<td>0.16; 0.11; 0.22</td>
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<td>0.29; 0.20; 0.42</td>
<td>0.14; 0.10; 0.20</td>
<td>0.14; 0.10; 0.21</td>
<td>0.09; 0.08; 0.14</td>
</tr>
</tbody>
</table>

* fever set to “yes” when T>40.0°C

## Odds ratio (reciprocal values) - Summarizing table

<table>
<thead>
<tr>
<th></th>
<th>fever</th>
<th>appet</th>
<th>cons</th>
<th>resp</th>
<th>nasal</th>
<th>cough</th>
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</thead>
<tbody>
<tr>
<td><strong>ID vs IM</strong></td>
<td></td>
<td></td>
<td>0.59</td>
<td></td>
<td>0.53</td>
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<tr>
<td><strong>C vs IM</strong></td>
<td>3.66</td>
<td>4.78</td>
<td>6.38</td>
<td>3.70</td>
<td>10.98</td>
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<tr>
<td><strong>C vs ID</strong></td>
<td>3.36</td>
<td>3.46</td>
<td>10.82</td>
<td>10.22</td>
<td>3.11</td>
<td>7.05</td>
</tr>
</tbody>
</table>

Only values for significant comparisons (chi-square, P<0.05) are reported.
Vaccine efficacy

- **DEFINITION:** The efficacy of the treatment is measured by the *proportion of cases* that it prevents.
  
  - Efficacy is directly calculated from the risk ratio (or rate ratio) comparing disease outcome in the treated versus control group.
  - For successful treatment this ratio will be < 1.
  - Efficacy = 1-RR
Results

Virology
PCR

Virus isolation
Virus titration

Results

Serology
Results

Cell mediated immunity
Conclusions

1. **70% of vaccine efficacy** (reduction of the duration and severity of clinical signs)
2. Short and **long term effect on BW**
3. Virology
   - **No differences in the percentage of viremic pigs** at any time
   - Some effect in term of duration of viremia (n.s.)
   - **No differences at virus quantitation** at any considered time
4. Anamnestic serological response
5. High levels of **IFN-γ SC** 14 to 27 days PE concurrently with the most consistent increase of **NK cells and cytotoxic T lymphocytes** in blood of vaccinated pigs can sustain the clinical protection.
Thank you

Notes
Intervet Satellite Symposium

Speaker: Stefano Gozio
Title: Porcilis PRRS, flexibility of the use with other Intervet vaccines

Stefano obtained his veterinary degree (cum Laude) in 1996 from the University of Parma in Italy. Stefano furthered his studies obtaining a post graduate degree in "Swine Pathology" from the University of Parma. In 1998 Stefano started a career as a vet practitioner in pig farms.

He joined Intervet Italia in the end of 2000 as Product Manager Swine. In April 2005 Stefano transferred to Intervet International as an International Product Manager for Swine.
Porcilis PRRS, flexibility of the use with other Intervet vaccines

Stefano Gozio
Intervet International
The Netherlands

Porcilis PRRS: wish list
• Safety and economic efficacy
• Flexible vaccination scheme:
  - Both individual and mass vaccination in breeding pigs
  - Both early and late vaccination in finishing pigs
• Flexible in use:
  - Can be combined with other Intervet vaccines

Porcilis PRRS & Porcilis M Hyo

Porcilis PRRS and Porcilis M Hyo vaccinations

<table>
<thead>
<tr>
<th>1 wk</th>
<th>2 wks</th>
<th>3 wks</th>
<th>4 wks</th>
<th>5 wks of age</th>
</tr>
</thead>
</table>

Porcilis PRRS in finishing pigs

Porcilis PRRS in breeding pigs

Thanks
Diluvac Forte!!!
Porcilis PRRS & Porcilis Porcoli DF

Materials and Methods

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Porcilis PRRS DF</th>
<th>Porcilis PRRS + Porcilis Porcoli DF</th>
<th>Porcilis Porcoli DF</th>
</tr>
</thead>
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<td>T0</td>
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<td>Porcilis PRRS + Porcilis Porcoli DF</td>
<td>Porcilis Porcoli DF</td>
</tr>
<tr>
<td>Vaccination and sampling</td>
<td>1st Serological test: ELISA E.coli and SP-IDEXX</td>
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</tr>
<tr>
<td>T1 (T0 + 4 wks)</td>
<td>Porcilis PRRS DF</td>
<td>Porcilis PRRS + Porcilis Porcoli DF</td>
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<tr>
<td>Vaccination and sampling</td>
<td>2nd Serological test: ELISA E.coli and SP-IDEXX</td>
<td></td>
<td></td>
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<tr>
<td>T2 (T0 + 8 wks)</td>
<td>Porcilis PRRS DF</td>
<td>Porcilis PRRS + Porcilis Porcoli DF</td>
<td>Porcilis Porcoli DF</td>
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<tr>
<td>Sampling</td>
<td>3rd Serological test: ELISA E.coli and SP-IDEXX</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 3 PRRS/AVD free gifts per group

Results

In vitro stability of the DV strain of Porcilis PRRS

In vitro stability: titration of the DV strain "Porcilis PRRS"

ELISA PRRS

Results

Seroconversion after vaccination
Conclusion

In our test system combined vaccination is equal to single administration

Thank you for your attention!!!
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of the 5th International Symposium
on Emerging and Re-Emerging Pig Diseases
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Materials and methods
Fifty herds showing respiratory and/or reproductive symptoms were sampled between 2004 and 2006. Clinical and epidemiological data were collected by using a questionnaire which comprised variables like herd size, animal flow, type of production, clinical signs and vaccination status. In addition, some of the herds were resampled one year later, and their status was recorded again. PRRSV isolates were analysed by RT-PCR amplification and sequencing of the highly variable ORF5. Alignments and phylogenetic analyses were calculated using the CLUSTAL X program. The alignments were optimised in BioEdit.

Results
The results of the questionnaire were as follows. In about 60% of the herds clinical signs became evident in autumn and winter. In about 50% of the herds only respiratory symptoms were recorded, and 38% had reproductive problems. In 4% of them unrelated clinical signs were observed, and the owners of 8% of the herds did not mention any typical clinical signs. Furthermore, 76% of the swine herds obtained their animals from one or two sources only, while 8% remounted from variable sources. The animals from 40 herds were vaccinated. From these, 15 herds used the EU attenuated vaccine, 19 herds the US attenuated vaccine, and 6 herds used an inactivated vaccine. From the herds where an attenuated vaccine was used, 10 switched the vaccine when clinical signs reappeared.

Most of the isolates were obtained from swine herds located in a close neighbourhood to other herds.
Virus was identified in 176 samples from the 50 herds. Most of the isolates (from 63% of the herds) were obtained from swine herds located in a close neighbourhood to other herds (distance < 3 km). One hundred virus isolates were sequenced, and the complete ORF5 sequences were used for phylogenetic analysis. EU field isolates were obtained from 24 herds, EU vaccine virus from 3 herds, and US vaccine derived virus from 15 herds. Seven herds harboured both the US derived vaccine virus and EU field isolates, and from one herd both vaccine viruses and EU field virus was isolated. Of the isolates from this study, 33 were of the US genotype, displaying nucleotide identities of 97-99% with the US live vaccine strain Ingelvac. The remaining isolates were of the EU genotype. They clustered into five subgroups, and four isolates were identical to the EU attenuated vaccine strain DV. Other EU isolates showed nucleotide identities to the Lelystad virus between 85 and 99%, to the EU vaccine strain DV between 86 and 98% and to the US vaccine strain between 58 and 62%.

Discussion and Conclusions

To minimize the effects of PRRSV infections on reproductive failure and respiratory distress producers vaccinate their herds either using an inactivated or a modified-live virus vaccine, based on or attenuated US or EU genotype virus. Most of the herds analysed in this study (34/50) used one of two live attenuated vaccines. Although both vaccines have been shown to effectively reduce clinical signs due to PRRSV infections, both vaccine virus strains have the capacity to persist and shed the virus for several weeks (Batista et al., 2002; Martelli et al., 2007). At least for the US-vaccine virus it was found that it could be transmitted to other pigs and to other herds (Lager et al., 2002). The fact that mainly US genotype virus was isolated from pigs also in farms which had stopped vaccination with the US attenuated vaccine one year or even longer before they were sampled supports the finding that the virus is not effectively eliminated by the immune system. Interestingly, the nucleotide sequences of the US genotype virus isolates from one herd were only to 96% identical to that of the US vaccine virus, and they formed a separated cluster in the phylogenetic tree. It remains to be elucidated whether these isolates are derived from the US-vaccine strain by multiple mutations, or if they are US genotype field isolates. In contrast, the four EU vaccine derived virus isolates were from herds that had been vaccinated with this vaccine shortly before sampling.

4 EU vaccine derived virus isolates were from herds that had been vaccinated with this vaccine shortly before sampling.

References


Efficacy of EU-PRRS Attenuated Vaccine to Control Respiratory Disease in Pigs Naturally Exposed to a Virulent Heterologous Field Virus

P. Martelli¹, S. Rosina¹, E. Bottarelli¹, S. Gozio², L. Ferrari¹, E. De Angelis¹, P. Borghetti¹

¹Department of Animal Health – University of Parma - Italy; ²Intervet International – Boxmeer – The Netherlands

Introduction and Objectives

Porcine Reproductive and Respiratory Virus (PRRSV) causes reproductive failure in sows and respiratory disorders in pigs of all ages, with consequent economic impact on the swine industry. Approximately 80% of the annual costs to the US pork industry of PRRS is estimated to be due to direct losses in growing and finishing pigs (4). The relative virulence, genetic divergence and presumably at least some antigenic diversity among PRRSV strains, all raise questions about immunity and cross-protection and the efficacy of the current European-type vaccines. Labarque at al. (1) showed a complete reduction in virus titres in lungs and blood of pigs vaccinated with an attenuated European-type vaccine subsequently challenged with homologous PRRSV isolates. The virological protection in vaccinated pigs experimentally exposed to a genetically divergent isolates (Italian strain) has been shown to be incomplete but significantly reduced, as compared to unvaccinated animals (2, 3). Information is scant about the immune response, and clinical as well as virological protection in vaccinated pigs subsequently exposed to natural infections under field condition. The present study was aimed at evaluating the clinical protection (efficacy) offered by a attenuated vaccine based on DV strain (Lelystad cluster) in pigs subsequently exposed to natural infection by an heterologous field strain of virulent PRRSV (Italian cluster). In addition, the more conventional intramuscular route of vaccination was compared with the intradermal route of vaccination.

Materials and methods

Thirty 4-week-old pigs from a PRRSV and Mycoplasma hyopneumoniae free herd were divided into three groups (10 pigs/group) designed IM, ID and C and housed in an isolation unit away from the farm of origin (Site 2 unit). At 5 weeks of age, groups IM and ID were vaccinated intramuscularly or intradermally, respectively, with Porcilis® PRRS (Intervet B.V. – The Netherlands) at a dose of 0.5 TCID₅₀ per pig. Group C pigs were left unvaccinated and served as controls. Intradermal vaccination was carried out using the IDAL® vaccinator. At day 5 post-vaccination (PV), (exposure day), pigs of all groups were moved to site 3, conventional finishing herd, mixed and in contact with resident pigs in the same building. The recipient herd (Site 3) had a history of respiratory disease caused by PRRSV along with the most common bacteria. In the two weeks preceding the exposure, any diseased and dead pigs in the recipient herd were subjected to microbiological and serological investigations. PRRSV was demonstrated by PCR and ORF5 sequence was performed as described by Oleksiewicz et al. (5). Pigs were monitored and scored daily for general and respiratory clinical signs throughout the post-exposure period (3 days). General Clinical Score (GCS) included: body (rectal) temperature (scored as 0: T ≤39.9°C; : T between ≥0.0 and ≤0.9°C; 2: T ≥10°C); appetite (0=normal or 1=anorexic); and level of consciousness (0=normal or 1=compromised). Respiratory Clinical Score (RCS) included a respiratory disease score ranging from 0 to 4 (0=normal, 1=tachypnoea when stressed, 2=tachypnoea at rest, 3=tachypnoea and dyspnoea at rest, 4=severe tachypnoea and dyspnoea with laboured, jerky breathing); coughing score (0=absent or 1=present); and nasal discharge score (0=absent or 1=present). Scores were totalled for each group. An Overall Clinical Score was calculated by adding the general clinical score (GCS) and the respiratory clinical score (RCS). Statistical analysis. Group scores were compared by Poisson regression. Vaccine efficacy was calculated according to Kirkwood and Stern (2003).
Results

At the nucleotide level (ORF5), the Italian virulent naturally field isolate was 84% homologous with the vaccine strain. Two pigs in the control group and one in the ID group died in the post exposure period and PRRSV and Pasteurella multocida were demonstrated in their lungs.

The course of the overall clinical score (mean values) per group during the observation period is shown in Figure 1.

Table 1: Statistical significance (p values) of the reduction in clinical scores

<table>
<thead>
<tr>
<th>General Clinical Score</th>
<th>Respiratory Clinical Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body T°C</td>
<td>Appetite</td>
</tr>
<tr>
<td>IM vs C</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>ID vs C</td>
<td>$&lt;0.0001$</td>
</tr>
</tbody>
</table>

With respect to the efficacy, the overall clinical signs were reduced by 68 and 72% respectively in the intramuscularly and intradermally vaccinated pigs compared to controls. Respiratory signs were reduced by 72% and 80%, respectively in the IM and ID groups. However, no statistically significant differences were apparent between the two routes of administration.

Discussion and Conclusions

Previous studies (2, 3) concluded that EU-PRRSV live vaccines confer some degree of a virological cross-protection to heterologous isolates of PRRSV as shown by significantly reduced virus titres in the blood or the respiratory tract of vaccinated challenged pigs.

In this study, evidence of EU attenuated vaccine-induced clinical protection against natural exposure to a genetically diverse (84% homology) PRRSV isolate (Italian cluster) was demonstrated by the statistically significant reduction in clinical signs in terms of incidence, duration and severity in vaccinated pigs as compared to the unvaccinated controls.

Statistically significant reduction in clinical signs in vaccinated pigs.

Acknowledgements

This work was supported by a grant from the University of Parma (Italy) - FIL 2006 – prot. 60A07-2704

References

(3) Martelli P, et al.. Vaccine 2007 (in press);
Materials and methods

This study was performed in 7 sow herds of different sizes and locations in Spain.

Table 1: Characteristics of the farms involved

<table>
<thead>
<tr>
<th>Farm</th>
<th>Number of sows</th>
<th>Type</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>1000</td>
<td>Closed cycle</td>
</tr>
<tr>
<td>B</td>
<td>2000</td>
<td>Three phases</td>
</tr>
<tr>
<td>C</td>
<td>1100</td>
<td>Three phases</td>
</tr>
<tr>
<td>D</td>
<td>800</td>
<td>Three phases</td>
</tr>
<tr>
<td>E</td>
<td>700</td>
<td>Three phases</td>
</tr>
<tr>
<td>F</td>
<td>700</td>
<td>Three phases</td>
</tr>
<tr>
<td>G</td>
<td>2000</td>
<td>Three phases</td>
</tr>
</tbody>
</table>

In the 7 cases the decision of using an attenuated PRRS vaccine was motivated by the detection of a reproductive course characterized by late abortions, higher number of weak born piglets and increasing levels of antibodies against PRRSv in the sows (PRRS virus antibody test kit – IDEXX Laboratories Inc. Westbrook, USA). A cross sectional serum profile (3, 5, 7 and 9 weeks of age) was carried out in the piglets of the five farms. The higher levels of positivity (S/P Ratios > 0.5) in these animals confirmed the incidence of early infections due, in part probably, to vertical transmission of the virus from the sows to their litters. This early infections were followed by high levels of mortality during the lactation and post-weaning periods.

Under these circumstances the vaccination was performed according to the following schedules:

• Farm A: Two herd vaccinations separated by a 4 weeks interval. Then the sows were vaccinated according to a 5/50 scheme (5 days of lactation and 50 days of pregnancy).
• Farm B: Two vaccinations at about 40 and 70 days of pregnancy. After farrowing the sows were vaccinated according to a 5/50 scheme
• Farms C and D: 5/50 scheme.
• Farms E, F and G: Herd vaccinations every 3 months.

The replacement stock was vaccinated two times with a 4 weeks interval during the quarantine period, receiving the second dose at least four weeks before entering the reproductive herd.

Porcilis® PRRS (Intervet International BV, Boxmeer, NL) is an attenuated live vaccine, based on a European strain of PRRS virus identified as DV strain.
Results and discussion

No undesirable effect was detected after the vaccination of the sows according to the different vaccination schedules.

In the seven cases, the piglets from the first sows vaccinated showed a clear reduction of the percentages of mortality during the lactation and post-weaning period. However we decided to follow up the piglets in order to associate this improvement of these parameters to any change in the patterns of circulation of the PRRS virus in this kind of animals. We monitored piglets of 3, 5, 7 and 9 weeks of age (5, 7 and 9 weeks in the farm E and 3, 5 and 7 weeks in farms F and G) and the results of the five serum profiles obtained are summarized in the Figure 1.

According to these data, the incidence of early infections in piglets from vaccinated sows was clearly reduced, compared to the former situation. However we still could detect two PRRS positive piglets at 9 weeks of age whose S/P ratios (<1.5) suggested us that this infection could be due to horizontal transmission of the virus from older pigs hosted in the same barn or an early infected piglet that we could not detect.

The incidence of early infections in piglets from vaccinated sows was clearly reduced.

Conclusion

The vaccination with Porcilis® PRRS used according to a wide range of vaccination schemes is an effective measure for controlling PRRS from a clinical and epidemiological point of view.

References

Materials and methods

Twenty-eight-day old pigs (n=30) from a conventional herd, seronegative and PCR negative for PRRSV and seronegative for Mycoplasma hyopneumoniae were used. At 5 weeks of age, pigs of group IM (n=10) and ID (n=10) were vaccinated intramuscularly (IM) and intradermally (ID) respectively with Porcilis® PRRS at a dose of $0.5 \times 10^{4.5}$ TCID$_{50}$ per pig. Pigs of group C (n=10) were kept unvaccinated and served as controls. Intradermal vaccination was performed by using the I.D.A.L.® needle-free vaccinator. After 5 days PV, the animals were moved to a site 3 conventional finishing herd and mixed with PRRSV-infected resident pigs. Blood samples were collected during the post-vaccination (PV) period at 0, 7, 14, 28, 45 days and in the post-exposure (PE) period at 0 (5 days PV), 7, 9, 2, 27, 3 days. Analysis of lymphocyte absolute values in blood was performed by flow cytometry as previously described (2) and the levels of specific IFN-γ Secreting Cells (SC) were determined by ELISpot assay using DV strain of PRRSV for in vitro stimulation of PBMC (Peripheral Blood Mononuclear Cells) (1). Statistical analysis was performed by ANOVA for repeated measures test, Dunnett test and Chi-square/Fisher-test. P values <0.05 were considered significant.

Results

The course of CD3-CD8α+ (NK cells) shows significantly higher levels at 28 and 45 days PV in both vaccinated groups in regard to controls; a further increase is observed at 19 days PE. In the PE period, the increasing trend of NK cells in ID-vaccinated pigs is significantly higher as compared to both IM-vaccinated and control pigs (Fig.1).
The course of cytotoxic T lymphocytes (CD4-CD8α+high) runs together with that of NK cells; in ID-vaccinated pigs, the increasing trend in the PV period is significantly different as compared to controls. After 19 days PE, cytotoxic T lymphocytes maintain high levels in both vaccinated groups, with no significant differences (data not shown).

At 28 and 45 days PV, IFN-γ PRRS-specific SC (Fig.2) show a significant increase in both vaccinated groups as compared to controls. Also in the PE period, both vaccinated groups show an increasing trend of IFN-γ SC, significantly different as compared to controls. Taken together, IFN-γ data during the PE period are not significantly different comparing the two vaccinated groups.

**Discussion and Conclusions**

Innate immune NK cells and conventional MHC-I restricted cytotoxic T lymphocytes play a pivotal effector role in antiviral immune response and protection; they are strongly able to proliferate and act as effector cells, killing infected cells and producing Th1 cytokines, especially IFN-γ (5). The maintenance in the PE period of high and constantly increasing levels of IFN-γ Secreting Cells (SC) in vaccinated pigs, especially in ID group, demonstrates that after natural infection by a heterologous virulent PRRSV strain, reactive cells to PRRSV persist in blood and are able to respond to in vitro stimulation. We can speculate that the increase of IFN-γ SC can be sustained by MHC-restricted T cells cross-reactive against two PRRSV strains or by an increase of activated innate cells (TCRγδ+ cells) or both (1, 3). The high levels of IFN-γ SC appear between 14 and 27 days PE concurrently with the most consistent increase of NK cells and cytotoxic T lymphocytes in blood and this can explain the clinical protection we have demonstrated in another paper. Particularly, after 19 days PE, ID-vaccinated pigs show higher levels of NK cells and cytotoxic T lymphocytes in blood. Data on blood lymphocytes show differences between vaccinated and control pigs we did not observe after experimental challenge (2); after natural infection, field conditions (e.g. coinfections or environmental stressors) can highlight the positive effect of vaccination on the production and recruitment of blood lymphocytes.

**Acknowledgements**

This work was supported by FIL 2006 prot. 60A07-2704 – University of Parma.

**References**

Introduction and Objectives

Due to the economical impact that PRRS has on a sow herd, eradication of porcine reproductive and respiratory syndrome virus (PRRSV) has become very important in swine production around the world. Today, several strategies to eliminate PRRSV are available, and the implementation of one or several of these strategies varies according to each farm situation. The objective of this study was to eradicate PRRSV from an unstable and active herd by mass vaccination and unidirectional pig and human flow.

Materials and methods

The selected farm was a 350 sow farrow-to-finish herd with a total inventory of 3,500 pigs ranging from 8 to 115 kg. The farm was located in a very low density pig area, and managed by all in/all out system. The farm had been infected with PRRSV at least for 10 years and presented clinical outbreaks of the diseases both in the sow and fattening units. Due to the economical impact that porcine reproductive and respiratory syndrome (PRRS) was having on the herd, in 2005 it was decided to implement an eradication strategy. The first step to eradicate PRRSV from the herd was to measure the stability of the sow herd and virus circulation in post-weaning (PW) and finishing areas (F). Polymerase chain reaction (PCR) results of pooled samples from weaning pigs showed that the sow herd was unstable (0 positive pools), and active circulation of PRRSV on the grower area was corroborated by results of IDEXX ELISA 2XR ((HerdChek PRRS ELISA; IDEXX Laboratories, Westbrook, Maine) of serum samples (5/0 and 5/5 positive samples from nursery and finishing pigs respectively).

The selected eradication protocol was based on unidirectional pig and people flow, and mass vaccination with Porcilis® PRRS (Intervet S.A., Beaucouzé, France), and shown in table 1.

<table>
<thead>
<tr>
<th>Week # (2005)</th>
<th>Vaccination protocol</th>
<th>Other measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>Vaccination on the same day of all sows and pigs in the PW and F stage</td>
<td>Herd closure and implementation of unidirectional pig and human flow</td>
</tr>
<tr>
<td>24</td>
<td>Booster vaccination of all vaccinated pigs on week 20, and vaccination of all weaned piglets since week 20</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>Booster vaccination of all vaccinated pigs (week 24) and vaccination of all other weaned piglets</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Implementation of piglet vaccination 2 and 6 weeks after weaning, until week 40</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>One dose vaccination of piglets two weeks after weaning until September of 2006</td>
<td>First entry of vaccinated gilts in the quarantine area</td>
</tr>
</tbody>
</table>
Results

After week 36, the sow herd became stable as corroborated by the 3-step system (1). Ten non-vaccinated piglets from 3 different batches (with one month intervals) were serologically monitored with the IDEXX ELISA test to evaluate the PW and F areas stability; results are shown on table 2.

| Table 2: Serology and PCR results from serum samples in growing pigs |
|-----------------|--------|--------|--------|--------|--------|
| Age (weeks)     | Batch  |
| Batch           | 11     | 15     | 19     | 23     | 27     |
| B 1             | 2/10   | -      | -      | 6/10   | PCR+   |
| B 2             | 2/10   | 1/10   | -      | -      | -      |
| B 3             | 2/10   | -      | -      | 3/10   | PCR+   |

(-)= negative, na= not available

On batches B1 and B3, two random pooled positive samples were sequenced (ORF7), and results presented 99.5% and 100% homology respectively with the vaccine strain. Therefore it indicated that field virus circulation had stopped; PCR positive results are consistent with a natural and limited vaccine viremia on “sentinel” pigs in direct, close contact with vaccinated pigs (2).

The estimated investment costs of this protocol was € 20,000 for vaccination, and € 500 for disinfection. However as presented on table 3, the improvement on production parameters paid for the extra cost and gave a total return on investment of € 64,100.

<table>
<thead>
<tr>
<th>Table 3: Production parameters before and after the implementation of PRRSV eradication protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSY</td>
</tr>
<tr>
<td>2005</td>
</tr>
<tr>
<td>2006</td>
</tr>
<tr>
<td>Gain in €</td>
</tr>
</tbody>
</table>

PSY= pigs per sow per year, PWFC= post-weaning feed conversion, FFC= finishing feed conversion, AM= age to market (days), % mort PW= % mortality in post-weaning, % mort F= % mortality in finisher

Discussion and Conclusions

Mass vaccination and unidirectional pig and human flow seem to be a valuable and cost benefit tool to eliminate PRRSV under the French production system.

However it must be noted that it is necessary to understand maternal immunity dynamics, and in case of any positive results, sequencing has to be performed to ensure that virus circulation of the field strain is not happening.

Mass vaccination and unidirectional pig and human flow is a valuable and cost benefit tool to eliminate PRRSV

Acknowledgements

The authors would like to thanks the farm owners for their cooperation in the implementation of the eradication protocol, and Intervet S.A. for their financial support.

References

**Introduction and Objectives**

Gilt introduction is a key step for controlling PRRS infection (5). A possible cause for persistent viral shedding within the breeding herd population is the introduction of naive or actively infected seedstock (3). For many veterinarians and specialists, introduction of PRRS-negative gilts into PRRS-positive herds is a must (5), but with an adapted quarantine period: these replacement gilts would need to be exposed to PRRS virus before entering the gestation facilities, in order to avoid a new wave of infection due to this negative subpopulation (1).

We introduce here a new concept, developed by a large pig company, allowing to perform safe adaptation of replacement gilts, in order to control PRRS infection in positive production herds. Preliminary results are presented as a first step to validate the protocol.

**Materials and methods**

The concept of introduction of replacement gilts into PRRS-positive production farms is based on 2 essential parameters (*Table 1*):

1. The stability/unstability of the production herd, according to the classification proposed by Dee (2).
2. The environmental risk of PRRSv (re)infection for the production herd.

According to these farm characteristics, one could advise, with individual agreement from the practitioner, recommendations about the origin of incoming gilts and the protocol of vaccination of these gilts during the quarantine period.

*Table 1: introduction of replacement gilts into PRRSv-positive production herds.*

<table>
<thead>
<tr>
<th>Production farm PRRS-status</th>
<th>Environment risk</th>
<th>Incoming gilts</th>
<th>Quarantine vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive very unstable</td>
<td>High</td>
<td>Prot. Vacc.</td>
<td>++++ MLV</td>
</tr>
<tr>
<td>Positive unstable</td>
<td>High/Moderate</td>
<td>Prot. Vacc.</td>
<td>+++ MLV</td>
</tr>
<tr>
<td>Positive stable</td>
<td>Moderate/High</td>
<td>Prot. Vacc.</td>
<td>+++ MLV</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>Prot. Vacc. or FREE</td>
<td>+++ MLV or KV</td>
</tr>
<tr>
<td>Negative</td>
<td>Low/Moderate</td>
<td>FREE</td>
<td>+ / - KV or 0</td>
</tr>
<tr>
<td></td>
<td>Low/Nil</td>
<td>FREE</td>
<td>- 0</td>
</tr>
</tbody>
</table>

MLV: Modified Live Vaccine; KV: Killed Vaccine; 0: No vaccine.

Free gilts (FREE) come from PRRSv-negative herds without any PRRS vaccination. Elisa serologies (IDEXX Herdcheck PRRS 2XR) are performed on 10 gilts every 3 weeks, on serum samples pooled by 5.

“Protected Vaccinated” gilts (Prot.Vacc.) come from “stabilized” PRRSv-positive herds, without PRRS related clinical signs, with implementation of a vaccination protocol based on the use of a modified live vaccine (MLV; Porcilis® PRRS from Intervet) on sows (blitz vaccination) and weaned piglets.
Individual Elisa serologies on 10 non vaccinated “sentinel” pigs are done every 3 weeks; a total of 6 vaccinated gilts (130-150 days of age) are also tested individually by PCR every 3 weeks. In most situations (except for negative production farms), vaccination of gilts with the PRRS MLV (Porcilis® PRRS) when entering the quarantine was advised, especially in unstable farms and/or with high to moderate environmental risk of infection.

**Results**

At this time, 10 farms have been included in the protocol. In all farms, preliminary results regarding clinical adaptation in quarantine are quite encouraging, as no clinical signs were registered. More detailed reproductive and health performances are available, when abstract in press, on 3 farms (Table 2).

<table>
<thead>
<tr>
<th>Farm</th>
<th>Farm size</th>
<th>Number of sows</th>
<th>Number incoming gilts (time)</th>
<th>Fertility (%)</th>
<th>Respiratory outbreaks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>350</td>
<td>50 (year)</td>
<td>150 (1 year)</td>
<td>94</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>150</td>
<td>55 (6 months)</td>
<td>30 (6 months)</td>
<td>90</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>250</td>
<td>55 (6 months)</td>
<td>55 (6 months)</td>
<td>95</td>
<td>No</td>
</tr>
</tbody>
</table>

**Discussion and Conclusions**

This protocol is an intermediate step for PRRS control within a farm or an area; it is well adapted to the situation of Brittany, where field strains only belong to European subtype and are relatively homologous as well between them than with the MLV strain (DV). For Dufresne (4), one of the key factors in the PRRSV control strategy is to introduce gilts that have already been exposed to the infection at a young age, so they have recovered, developed immunity and are not shedding the virus: it could be also achieved by PRRS vaccination of gilts before entering the production herd. If PRRS elimination is the goal, then it would be better to bring naïve replacement animals (4). But herds are vulnerable to re-infection with PRRS virus especially in widely contaminated areas with high pig farm density, via currently unidentified routes of entry (3). Introduction of gilts vaccinated at 6 weeks of age (produced if possible on a separate site and under a strict monitoring) into a positive herd could be, with effective 24 weeks MLV (Porcilis® PRRS) protection, one tool to reduce significantly the risk of spreading and destabilization of the field PRRSV within the herd, before starting a real eradication strategy.

**References**

Materials and methods

This field trial was settled in a farrow-to-finishing farm (230 sows) located in Côtes d’Armor (Brittany), with a 3 weeks batch AI/AO management, serologically PRRS positive. Incoming gilts are purchased from a PRRS-free selection herd.

One batch of gilts included in the study (a total of 17 animals) was vaccinated intradermally (0.2 ml in the perivulvar area) when entering the quarantine with Porcilis PRRS IDAL (Intervet), then blood sampled 4 weeks later.

A complete batch of 290 weaned piglets (6 weeks of age) was selected, located in 2 rooms, divided in groups (one compartment in each room for each group):

- Group 1: control group: no vaccination,
- Group 2: IM neck: one intramuscular injection of Porcilis PRRS IM (2 ml) in the neck,
- Group 3: IDAL neck: one intradermal injection of Porcilis PRRS IDAL (0.2 ml) in the neck,
- Group 4: IDAL loins: one intradermal injection of Porcilis PRRS IDAL (0.2 ml) on the back (loins).

The used vaccines for this field trial were Porcilis PRRS IM (50 doses vials) and Porcilis PRRS IDAL (100 doses vials) (Intervet).

Blood sampling has been done on a total of 70 piglets (10 animals in group 1; 20 piglets in each other group), 4 weeks after inclusion in the study and injection.

Laboratory analysis done on sera were an Elisa PRRS test (2XR Idexx kit, cut-off level ratio S/P: 0.4), performed in L.D.A. 22 laboratory (Ploufragan, France), and an IFA test (European PRRSv strains, cut-off level ratio: 4 log) done in R. and D. laboratory (Intervet International, Boxmeer, The Netherlands). Evaluation of safety has been done on gilts and weaned piglets from the unique vaccination till 7 days after injection.

Statistics have been performed with SYSTAT software.

Results

No systemic nor local reactions were observed on gilts and weaned piglets in any group.

Dealing with serologies, 17 gilts were sampled and analyzed in both serologies. 88 (IFA) and 100 % (Elisa) of these gilts did have a seroconversion 4 weeks after vaccination (Table 1).

Some handling problems were encountered during the intradermal vaccination, as it was a first use and practice with such method.

For both methods, the percentage of “responders” (positive with post-vaccinal response) was statistically different between the 4 groups (p ≤ 0.001), but not between the vaccinated groups (Table 2).
The Elisa titers (means and Standard Deviations), calculated on all piglets, were also not statistically different between the vaccinated groups (p = 0.433); only a high significant difference was found between the 3 vaccinated groups and the control group (p ≤ 0.001) (Table 2). IFA titers were significantly higher for the 3 vaccinated groups, compared to the control group (p < 0.001); then there was a significative difference between groups 1 and 3 (p = 0.029) (Table 2). Although IFA method is not linear, correlation was relatively high (r² = 0.873) and significative (p < 0.001) between the 2 serological methods.

Table 1: PRRS serologies in gilts.

<table>
<thead>
<tr>
<th>Nr gilts</th>
<th>Antibody response</th>
<th>Titer Elisa Idexx (S/P)</th>
<th>Titer IFA Intervet (log₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>% positive</td>
<td>100</td>
<td>88</td>
</tr>
<tr>
<td>17</td>
<td>Mean titer</td>
<td>1.82</td>
<td>9.0</td>
</tr>
<tr>
<td>17</td>
<td>Standard Deviation</td>
<td>0.74</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Table 2: PRRS serologies in piglets.

<table>
<thead>
<tr>
<th>Nr piglets</th>
<th>Group</th>
<th>Titer* Elisa Idexx: mean +/- SD (% positive)</th>
<th>Titer IFA** Intervet (log₂): mean +/- SD (% positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1</td>
<td>0.05 +/- 0.07 (0)</td>
<td>4 +/- 0 (0)</td>
</tr>
<tr>
<td>20</td>
<td>2</td>
<td>1.87 +/- 0.99 (70)</td>
<td>9.75 +/- 1.65 (80)</td>
</tr>
<tr>
<td>20</td>
<td>3</td>
<td>1.85 +/- 0.89 (70)</td>
<td>9.07 +/- 1.59 (70)</td>
</tr>
<tr>
<td>20</td>
<td>4</td>
<td>2.17 +/- 1.04 (50)</td>
<td>9.11 +/- 1.69 (45)</td>
</tr>
</tbody>
</table>

SD: Standard Deviation
* : S/P ratio
** : ImmunoFluorescence Assay

Discussion and Conclusions

These field serological data confirmed previous trials showing comparable response between intradermal and intramuscular applications with Aujeszky (5) or PRRS vaccination (4), both with the IDAL injector.

For PRRS vaccination, manipulation and handling of piglets could be better performed with the recent authorization for Porcilis PRRS vaccine about the required minimal age (from 2 weeks of age) (3,4). Therefore, before and at weaning, piglets could be individually handled to perform ID vaccination, so in better conditions than applied for the present field trial (collective vaccination applied within compartments of around 35 to 40 piglets).

The absence of tissue irritation using Diluvac Forte adjuvant and use of virus strain DV is in line with modern requirements for animal’s welfare and meat quality.

References

Introduction

PRRSv infection was described for the first time in France at the end of 1991. Vaccination with a modified live vaccine (MLV) based on an European strain (Porcilis® PRRS, Intervet) against PRRS is authorized in France since 2002 (growing pigs) and 2005 (reproductive animals). PRRS stabilization or eradication protocols using Porcilis® PRRS mass vaccination were already described in France (2,3). The purpose of this study is to present a mass vaccination protocol with a modified live vaccine in order to stabilize PRRS unstable farrow-to-finish herds.

Materials and methods

Two farrow-to-finish farms of respectively 180 and 350 sows, located in Brittany (the main pig production area in France), were selected for the study. The first one was infected in 1994 and the second one more recently in 2002. Because of unstability in the sow herd and active circulation in the finishing units, the vet from the pig organization decided in 2004 to implement mass vaccination in these two herds. First an inactivated vaccine (KV) was used in sows and the MLV (Porcilis® PRRS) was implemented on piglets. In 2005, after the authorization of the MLV on reproductive animals, the PRRS KV was replaced by Porcilis® PRRS on gilts and sows because of the plain PRRSv circulation still occurring on the “sentinels”.

The vaccination protocol established in the 2 farms from mid-2005 with Porcilis® PRRS was:
- Gilts: one injection when delivered to the farm (beginning of the quarantine).
- Sows: “blitz” mass vaccination 3 times a year (one injection every 4 months).
- Piglets: one injection between 5 and 6 weeks of age.

The introduction of replacement gilts was stopped for a period of 4 months, starting in June 2005. Biosecurity measures were also implemented in both farms, in terms of management of manure, conditions of circulation of animals within each farm, intensification of cleaning and disinfection protocols.

Around 6 months after the “blitz” mass vaccination, a monitoring of stability in sow herd and of virus circulation in post-weaning and finishing units started in both farms on the following basis:
- PCR test on 9 (from December 2005 to May 2006), then 6 vaccinated growing pigs per batch, at an age of around 140 days, on a 3-weeks rhythm. The sera were pooled by 3 before testing.
- Elisa individual serologies (IDEXX Herdcheck PRRS 2XR) on a minimum of 10 “sentinel” piglets (not vaccinated), every 3 weeks.

All the samples analysis were performed in L.D.A. 22 (Ploufragan, France).

Results

No serious adverse systemic and local reactions were observed during the vaccination series. No clinical signs were registered in both farms since the beginning of the vaccination period. Serology and PCR testing done before implementation of the mass vaccination protocol (first half of 2005) confirmed field PRRSv infection in both herds (Table 1). From November 2005 till mid December 2006, a total of 213 (119 in farm A and 94 in farm B) and 188 (99 in farm A and 89 in farm B) growing pigs has been controlled respectively by PCR and Elisa. After implementation of the mass vaccination, PRRS Elisa and PCR results were found “negative” on both farms (Table 1).
**Table 1: evolution of serological results (PCR, Elisa) on growing pigs in farms A and B.**

<table>
<thead>
<tr>
<th>Time</th>
<th>PCR / Elisa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Farm A</td>
</tr>
<tr>
<td>Feb.2005</td>
<td>pos/ND</td>
</tr>
<tr>
<td>Mar.2005</td>
<td>ND/pos</td>
</tr>
<tr>
<td>May 2005</td>
<td>ND/pos</td>
</tr>
<tr>
<td>Jun.2005</td>
<td>ND/neg</td>
</tr>
<tr>
<td>Nov.2005</td>
<td>ND/ND</td>
</tr>
<tr>
<td>Dec.2005</td>
<td>neg/ND</td>
</tr>
<tr>
<td>Jan.2006</td>
<td>neg/ND</td>
</tr>
<tr>
<td>Feb.2006</td>
<td>ND/ND</td>
</tr>
<tr>
<td>Mar.2006</td>
<td>neg/ND</td>
</tr>
<tr>
<td>Apr.2006</td>
<td>neg/neg</td>
</tr>
<tr>
<td>May 2006</td>
<td>ND/neg</td>
</tr>
<tr>
<td>Jun.2006</td>
<td>neg/neg</td>
</tr>
<tr>
<td>Jul.2006</td>
<td>ND/neg</td>
</tr>
<tr>
<td>Aug.2006</td>
<td>neg/neg</td>
</tr>
<tr>
<td>Sep.2006</td>
<td>neg/neg</td>
</tr>
<tr>
<td>Oct.2006</td>
<td>neg/neg</td>
</tr>
<tr>
<td>Nov.2006</td>
<td>neg/ND</td>
</tr>
<tr>
<td>Dec.2006</td>
<td>neg/ND</td>
</tr>
</tbody>
</table>

ND: Not Done, neg: negative, pos: positive

**Discussion and Conclusions**

Results clearly show that field virus circulation is quite stabilized in the 2 farms since November 2005. In spite of missing data, all tested animals were negative by PCR and all “sentinel” pigs remain also negative. Even if it is too early to definitely claim for PRRSv eradication in both farms, the protocol applied with this MLV allowed to drastically reduce field virus circulation, beside the whole control of clinical signs. These field data confirmed the efficacy of Porcilis® PRRS to reduce significantly the viral excretion after infection (1).

This study showed that mass vaccination with a MLV is a convincing tool to stabilize pig herds in PRRS control programs. Stabilization of pig farms could be the first required step for a PRRS eradication plan in low density areas, but also a long-term way to control efficiently the PRRS infection in production herds within high density areas.

**References**

A FIELD TRIAL IN FRANCE TO ASSESS THE SAFETY AND THE EFFICACY AGAINST MYCOPLASMA HYOPNEUMONIAE OF SIMULTANEOUS USE OF PORCILIS PRRS AND PORCILIS M HYO IN PIGLETS

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Introduction and Objectives

In this clinical study, the safety and efficacy (against Mycoplasma hyopneumoniae) in pigs of simultaneous administration of Porcilis M Hyo and Porcilis PRRS was assessed in comparison with a group that received only Porcilis M Hyo (positive control group) and an unvaccinated control group (negative control group).

Materials and methods

Porcilis M Hyo is an inactivated vaccine against Mycoplasma hyopneumoniae, containing alpha-tocopherol as adjuvant. Porcilis PRRS is a live vaccine against Porcine Reproductive and Respiratory Syndrome (PRRS). For simultaneous use the freeze-dried PRRS was dissolved in the aqueous Porcilis M Hyo just prior to use.

The trial was designed as a partially blinded, randomised, controlled field trial and was conducted on two farrow-to-finish farms in France. Both farms were serologically positive for PRRS and on both farms an infection with M. hyopneumoniae was present, as confirmed by lung lesions at slaughter.

In total 991 piglets were selected for the study. The piglets were randomly divided into three groups and treated according to the schedule in Table 1. The vaccines and placebo were administered intramuscularly.

<table>
<thead>
<tr>
<th>Vaccination</th>
<th>Group</th>
<th>Group</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 ± 3 days</td>
<td>Porcilis M Hyo</td>
<td>Porcilis M Hyo</td>
<td>Control (n=330)</td>
</tr>
<tr>
<td>(weaning)</td>
<td>Porcilis M Hyo + Placebo</td>
<td>Porcilis M Hyo + Porcilis PRRS</td>
<td>-</td>
</tr>
</tbody>
</table>

The animals were monitored closely from 3 days before until 14 days after second vaccination. Injection site reactions, temperature (on 10 selected animals per group and per farm), feed intake, and general health were used as parameters for safety.

To determine vaccine efficacy, the lungs of the pigs were examined at slaughter and scored for specific lesions of M. hyopneumoniae infection according to the method of Goodwin and Whittlestone. The animals were weighed at weaning, at transfer to the fattening unit and before slaughter to determine their weight gain and also the mortality (total and due to respiratory disease) was recorded between weaning and slaughter.

Statistics:

The individual animal was the statistical unit. Descriptive statistics were used to present and summarise the results. First the effects of the simultaneous use of Porcilis M Hyo and Porcilis PRRS against Mycoplasma hyopneumoniae infections was evaluated by comparing the results of the M Hyo+PRRS group and the group vaccinated with M Hyo and placebo. Then the efficacy and safety data of the M Hyo and M Hyo+PRRS groups were combined and compared with the unvaccinated control group.

Proportions were tested in contingency tables using Cochran Mantel Haenszel methods. The lung lesion scores, being ordinal data, were first rank-transformed and then analysed by ANOVA with treatment and farm as explanatory variables. Weight gain and rectal temperature were analysed by ANCOVA with pre-treatment value as co-variable and farm and treatment as explanatory variables. Statistics were performed using SYSTAT software.
Results

Safety:
In up to 3.1% of the piglets from the Porcilis M Hyo and M Hyo+PRRS a minor and transient local reaction could be observed on the day after vaccination. These reactions consisted of a diffuse swelling and/or redness of the skin. By day 4 all reactions had disappeared. No reactions were observed in the unvaccinated group.

No systemic reactions or effects on the rectal temperature were observed except for an immediate reaction in one piglet after the first Porcilis M Hyo injection. The animal dropped down, showed erythema and an increased respiration rate during about 3 minutes and then recovered rapidly and uneventfully.

Efficacy:
The results of the lung lesions scoring are summarised in Table 2. No significant differences between the M Hyo and M Hyo+PRRS vaccinated groups were observed.

Vaccination with Porcilis M Hyo reduced the proportion of pigs with pneumonic lesions at slaughter from 53% in the control group to 33% in the vaccinated pigs (Cochran Mantel Haenszel test: p <0.001). The mean lung lesion score was also significantly reduced: from 3.74 ± 5.9 in the unvaccinated piglets to 1.74 ± 4.08 in the piglets vaccinated with Porcilis M Hyo (ANOVA: p < 0.001).

Table 2: Results of lung lesion scores; by vaccination group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Pneumonic lesions</th>
<th>Mean lesion score</th>
</tr>
</thead>
<tbody>
<tr>
<td>M Hyo+PRRS</td>
<td>95 / 265 (36%)*</td>
<td>1.88 ± 4.4</td>
</tr>
<tr>
<td>M Hyo</td>
<td>81 / 262 (31%)</td>
<td>1.63 ± 3.9</td>
</tr>
<tr>
<td>Control</td>
<td>144 / 270 (53%)</td>
<td>3.79 ± 5.9</td>
</tr>
</tbody>
</table>

*number of lungs with lesions / total number of lungs scored (%)

Table 3: Average daily gain (g/day); by period and vaccination group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Post-weaning period</th>
<th>Fattening period</th>
<th>Weaning - slaughter</th>
</tr>
</thead>
<tbody>
<tr>
<td>M Hyo+PRRS</td>
<td>437 ± 68*</td>
<td>776 ± 120</td>
<td>638 ± 83</td>
</tr>
<tr>
<td>M Hyo</td>
<td>442 ± 69</td>
<td>777 ± 112</td>
<td>641 ± 78</td>
</tr>
<tr>
<td>Control</td>
<td>445 ± 67</td>
<td>759 ± 121</td>
<td>631 ± 83</td>
</tr>
</tbody>
</table>

*mean and standard deviation

No significant difference in ADG was observed between the M Hyo and M Hyo+PRRS vaccinated groups during any of three periods. However, vaccination with Porcilis M Hyo significantly (ANCOVA: p < 0.05) improved the ADG during the fattening period (from 759 grams per day in the control group to 776 grams per day in the vaccinated pigs). No statistically significant effects were found for the other periods. The results are summarised in Table 3.

Discussion and Conclusions

Neither the percentage of pigs with lung lesions nor any of the other efficacy and safety parameters showed a significant difference between the M Hyo and M Hyo+PRRS vaccinated groups. It can, therefore, be concluded that simultaneous vaccination with Porcilis M Hyo and Porcilis PRRS is as safe and efficacious as vaccination with Porcilis M Hyo only.

Vaccination with Porcilis M Hyo significantly reduced the incidence of pneumonic lesions and the mean lung lesion score. Weight gain is negatively influenced by lung problems. Vaccination with Porcilis M Hyo, therefore, improved the ADG during the fattening period, which is the period when pigs have the highest weight gain.
**Introduction**

The porcine respiratory disease complex (PRDC) is considered a major cause of losses for pig producers. The porcine respiratory and reproductive virus (PRRSV) and *Mycoplasma hyopneumoniae* (MHYO) are two of the most important pathogens involved in the PRDC. According to this, vaccination against both pathogens should be indicated, but it has been shown that the presence of PRRSV, either wild type US virus or the US MLV vaccine, significantly reduced the efficacy of MHYO vaccination, thereby limiting apparently the possibilities of vaccination against PRDC (2). However, a recent study found that an EU PRRS MLV vaccine (Porcilis® PRRS- Intervet International BV, Boxmeer, NL) did not reduce the efficacy of a MHYO vaccine (Porcilis® M Hyo- Intervet International BV, Boxmeer, NL) (1,4).

The aim of this paper is to confirm under field conditions the non-interference of Porcilis® PRRS on Porcilis® M Hyo.

**Materials and methods**

This study was performed in a closed cycle herd of about 20 sows located in the province of Huesca, in the north-east of Spain. Before the introduction of the simultaneous administration of Porcilis® PRRS and Porcilis® M Hyo, the piglets of this farm were vaccinated with another MHYO bacterin at one and four weeks of age with, apparently, good results (clinical signs, incidence and severity of lung lesions). This was a conventional bacterin with Al(OH)₃ as adjuvant.

In April of 2006 a circulation of PRRSV was detected in the nursery, that translated into higher rates of mortality. This PRRSV circulation was confirmed by a cross sectional serum profile (PRRS virus antibody test kit – IDEXX Laboratories Inc. Westbrook, USA).

Under these circumstances, it was decided to vaccinate the sows with Porcilis® PRRS (herd vaccination every 4 months). Replacement gilts were also vaccinated and revaccinated with Porcilis® PRRS. In September 2006, although PRRSV circulation at nursery was controlled, there were still high rates of mortality in the fattening units, related to PRRSV infection, as was confirmed by serology. Now it was decided that the piglets at four weeks of age be vaccinated with Porcilis® PRRS. Due to the fact that at that age the piglets were vaccinated against MHYO, to make the piglets vaccination easier, the substitution of the former MHYO bacterin by Porcilis® M Hyo was decided. It was administered according to the same vaccination scheme but in the second vaccination (at four weeks of age) Porcilis® M Hyo was used as diluent of the freeze-dried vaccine Porcilis® PRRS.

The effect of Porcilis® PRRS on the efficacy of Porcilis® M Hyo was evaluated through the presence and severity of lung lesions at slaughter associated to enzootic pneumonia according to the method described by Goodwin and Wittlestone (1967) with a maximum score of 55 points per lung. The lung lesion score obtained in the pigs vaccinated with Porcilis®PRRS+Porcilis®M Hyo was compared to the one obtained in pigs vaccinated with the former MHYO bacterin.
**Results and discussion**

No undesirable effect was detected after the simultaneous administration of both vaccines. According to the farmer, the performance of the piglets that received both vaccines simultaneously was improved and showed a clear reduction of the percentage of mortality during the fattening period.

In total 245 lungs were scored for lesions at the slaughterhouse. The results of this study are summarized in *table 1*.

*Table 1: Lung lesion score (LLS) at slaughter*

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Nº of pigs</th>
<th>LLS</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHYO bacterin</td>
<td>125</td>
<td>12.88</td>
<td>8.09</td>
</tr>
<tr>
<td>Porcilis®PRRS+</td>
<td>120</td>
<td>7.23</td>
<td>6.09</td>
</tr>
<tr>
<td>Porcilis®M Hyo</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The better results in terms of mortality rate obtained in the pigs vaccinated with Porcilis®PRRS+Porcilis®M Hyo suggests that this combination is effective in cases of PRDC, when PRRSV and MHYO are involved. Although the two MHYO bacterins were different, the lower LLS obtained in the pigs vaccinated with Porcilis®PRRS+Porcilis®M Hyo, could be considered to be an indication that simultaneous administration of these two vaccines does not reduce the efficacy of the MHYO bacterin.

**Conclusion**

The simultaneous administration of Porcilis® PRRS and Porcilis® M Hyo not only does not affect negatively the efficacy of the second vaccine, but is also an effective measure in cases of PRDC where PRRSv and MHYO are involved.

**References**

1. Drexler, C. et al. (2006) 18th IPVSV Congress
THE EFFECTS OF SIMULTANEOUS VACCINATION WITH PORCILIS PRRS AND PORCILIS M HYO IN PIGS

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Danish Pig Production / Danish Meat Association Axeltorv 3, 1609 København V

Introduction and Objectives

Infections with both *Mycoplasma hyopneumoniae* and *Porcine Reproductive and Respiratory Syndrome* virus (PRRSV) are widespread in Danish fattening herds. Hence, concurrent infections will occur in a number of herds, where the possibility that one infection potentates the other is present. Vaccines for both infectious agents are commercially available in Denmark, but no vaccines are approved for simultaneous application. However, a combined use of Porcillis® M Hyo and Porcillis® PRRS may provide an improved effect on respiratory disease caused by dual infections with *Mycoplasma hyopneumoniae* and PRRSV. The effect of dual vaccination has been investigated under experimental conditions with equivocal results (1,2). However, experiments indicate that pigs are able to develop a protective immune response in face of the presence of PRRSV (3).

The purpose of this trial was to determine any additive effect of simultaneous vaccination of piglets with Porcillis® M Hyo and Porcillis® PRRS.

Materials and methods

The effect was measured by health and production parameters in three fattening herds suffering from infections with *Mycoplasma hyopneumoniae* and EU-type PRRSV infection. In each herd the suckling pigs of at least one week of age, and three weeks prior to weaning, were included in the trial. The pigs were ear tagged with individual numbers and randomly allocated to one of four treatment groups - *table 1*.

*Table 1: The relations between treatments and treatment groups*

<table>
<thead>
<tr>
<th>Group</th>
<th>Placebo</th>
<th>PRRS</th>
<th>M Hyo</th>
<th>Combi</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Treatment</td>
<td>Diluvac Forte</td>
<td>Diluvac Forte</td>
<td>Porcillis M Hyo</td>
<td>Porcillis M Hyo</td>
</tr>
<tr>
<td>2. Treatment</td>
<td>Diluvac Forte +FDP</td>
<td>Diluvac Forte +Porcillis PRRS</td>
<td>Porcillis M Hyo +FDP</td>
<td>Porcillis M Hyo +Porcillis PRRS</td>
</tr>
</tbody>
</table>

*FDP = freeze-dried placebo.*

The pigs were vaccinated with either Porcillis M Hyo or Diluvac Forte (adjuvant) at 4-11 days of age (1.treatment). At weaning three weeks after first treatment the pigs were moved to nursery units and weighed. The pigs were vaccinated the second time (2.treatment) with either Porcillis M Hyo + freeze-dried placebo (M.hyo), Porcillis M Hyo + Porcillis PRRS (combi), Diluvac Forte + Porcillis PRRS (PRRS) or Diluvac Forte + freeze-dried placebo (placebo) according to *table 1*. The pigs were housed with pen mates belonging to the same treatment group for at least four weeks. This was done to prevent interaction between treatment groups.

During the observation period from weaning until slaughter the number of antibiotic treatments and the indication for antibiotic treatments was recorded. The number of dead pigs in total and death due to respiratory disease was recorded as well. At the end of the observation period the individual pigs were weighed again. At slaughter all lungs were examined for the presence and severity of enzootic pneumonia.
The primary effect parameter was average daily gain (ADG) and the prevalence and severity of lung lesions. Antibiotic treatment frequency (ATF) and mortality (MRD) during the observation period came as secondary effect parameter.

Sample size in each herd was calculated to be 1000 pigs. Based upon a power of 0.8 and a significance level of 0.05 this sample size aimed at showing an increase in ADG of 30 grams per day in each vaccine group when compared to placebo, and a 50% reduction in the prevalence of animals with pneumonic lung lesions in any given vaccine group compared to the placebo group.

### Results

#### Table 2: Average daily gain and (standard deviation) in grams

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Herd 1 ADG</th>
<th>N</th>
<th>Herd 2 ADG</th>
<th>N</th>
<th>Herd 3 ADG</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>M Hyo</td>
<td>762(81)</td>
<td>255</td>
<td>687(64)</td>
<td>218</td>
<td>724(80)</td>
<td>132</td>
</tr>
<tr>
<td>Combi</td>
<td>768(82)</td>
<td>258</td>
<td>673(79)</td>
<td>223</td>
<td>727(82)</td>
<td>137</td>
</tr>
<tr>
<td>PRRS</td>
<td>765(73)</td>
<td>257</td>
<td>670(81)</td>
<td>228</td>
<td>726(74)</td>
<td>134</td>
</tr>
<tr>
<td>Placebo</td>
<td>759(89)</td>
<td>259</td>
<td>679(78)</td>
<td>241</td>
<td>714(86)</td>
<td>135</td>
</tr>
<tr>
<td>Total</td>
<td>1.029</td>
<td>910</td>
<td>908</td>
<td>538</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: The prevalence of enzootic pneumonia (number of pigs)

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Herd 1</th>
<th>Herd 2</th>
<th>Herd 3</th>
<th>Herd 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>M Hyo</td>
<td>30(76)b</td>
<td>34(78)b</td>
<td>36(74)</td>
<td>36(73)</td>
</tr>
<tr>
<td>Combi</td>
<td>31(76)b</td>
<td>45(98)</td>
<td>37(84)</td>
<td>37(83)</td>
</tr>
<tr>
<td>PRRS</td>
<td>49(115)</td>
<td>237</td>
<td>206</td>
<td>206</td>
</tr>
<tr>
<td>Placebo</td>
<td>54(138)</td>
<td>255</td>
<td>233</td>
<td>233</td>
</tr>
<tr>
<td>Total</td>
<td>990</td>
<td>884</td>
<td>538</td>
<td>538</td>
</tr>
</tbody>
</table>

### Discussion and Conclusions

The trial showed that the effect of the vaccines was very much related to herd factors. Herd 3 did not contribute with the decided sample size and no significant effect was shown probably due to lack of power.

Keeping in mind that the pigs entered the trial at 4-5 weeks of age, there was no benefit to AGD in any of the vaccination groups compared to placebo – table 2. There was a reduction in the prevalence of pneumonic lung lesions, when using M.hyo or the combi vaccination – table 3. The severity of pneumonic lung lesions was reduced in the M.hyo group alone – table 4. No differences in ATF or MRD were seen between the vaccination groups in any of the herds.

It can be concluded that vaccination with Porcillis® M Hyo and Porcillis® M Hyo + Porcillis® PRRS can give some protection against Mycoplasma hyopneumoniae infection. No additive effect of concurrent vaccination of piglets with Porcillis® M Hyo and Porcillis® PRRS was seen.

### References

Materials and methods
The initial study investigated the stability of the PRRS vaccine strain when reconstituted in its normal (adjuvanted) Diluvac Forte (DF) diluent compared to when it was reconstituted with Porcilis Porcoli DF.

The in vitro stability over three hours was measured as titer log10 /ml. In the second study three groups of 8 gilts pigs were each injected on two occasions with either Porcilis Porcoli DF alone, Porcilis Porcoli DF and Porcilis PRRS or with Porcilis PRRS alone, by intramuscular injection. Blood samples were taken at T0, when the first vaccination was administered, at T1 (T0 plus 4 weeks) at the time of the second vaccination, and at T2 (T0 plus 8 weeks). PRRSV titer was measured by S/P (Idexx PRRSV) and E. coli titers were measured by ELISA.

Statistical evaluation was as follows: The titers between Porcilis Porcoli DF and the other two groups was analyzed by two-sample T-test with significance set at p<0.05
**Results**

There was no difference in the in vitro stability of PRRSv when suspended in either diluent (Fig. 1).

Figure 1: In vitro stability: titration of the Porcilis PRRS +DF against Porcilis PRRS in Porcoli DF

There was no significant difference in PRRSv titer between the PRRS + DF and PRRS + Porcoli DF (Fig. 2).

Figure 2: Vaccine efficacy according to the administration route (white bars= 95% Confidence Intervals)

Seroconversion to all E. coli antigens was acceptable, with end titers above the average protective level (log2 serum antibody titer of 10 or above) and no significant differences between the groups vaccinated with Porcoli DF or Porcoli DF+PRRS, data not shown.

**Discussion and Conclusions**

The data presented herein demonstrates that there is no loss of stability or efficacy in engendering more than adequate protective seroconversion when mixing and administering these two vaccines together.

**References**


Gozio et al. IPVS 2006 Safety and efficacy of combined administration of Porcilis PRRS and Porcilis Ery+Parvo

Gozio et al. IPVS 2006 Safety and efficacy of combined administration of Porcilis PRRS and Porcilis Begonia
**Introduction and Objectives**

Vaccination against Porcine Reproductive and Respiratory Syndrome (PRRS) with attenuated vaccines seems to be one of the most effective methods to control this infection (1). Vaccination against *Mycoplasma hyopneumoniae* (*M hyo*) infections with Porcilis® M Hyo (Intervet) has been proven to improve growth and lung lesions situation on the field (2). Vaccination against *Actinobacillus pleuropneumoniae* (*App*) infections is indicated in growing pigs to prevent respiratory outbreaks and lung lesions (as pleuritis) in slaughterhouse. Vaccination of weaned piglets with the subunit vaccine Porcilis® APP (Intervet) increases many health and zootechnical parameters in field conditions (3).

In PRDC (Porcine Respiratory Disease Complex) situations, the most important difficulty to implement a vaccination scheme involving PRRS, *M hyo* and *App* vaccines is the workload and the compliance. The objectives of this study were to get a first evaluation, in field conditions, of safety and efficacy (evaluation of serological response) of simultaneous use (mixing) of three Intervet vaccines which are based on the same adjuvant (Diluvac Forte).

**Materials and Methods**

The study was performed in a post-weaning and fattening herd located in the eastern part of France (Ain department). Every three weeks it received 272 piglets from mostly one farrowing herd, which is free of *App* and PRRS, and where the sows are vaccinated against PCV2. The piglets weight is about 8 kg at 28 days old. Historically the piglets were vaccinated against *M hyo* at 4 weeks of age (the day after incoming).

At the beginning of 2005, *App* biovar 1 serovar 2 was isolated from condemned lungs at slaughter. The losses due to a high level of condemnation for respiratory lesions lead to implement an *App* vaccination with the subunit vaccine Porcilis® APP (Intervet).

In May 2006, growth performances were good but pleuritis and condemnations at slaughter were persisting in spite of a good response to the vaccination (scheduled at 7 and 11 weeks) evaluated at 5 weeks by serology: Elisa tests Apx I, Apx II, Apx III and OMP performed by R&D Laboratory (Intervet International bv, Boxmeer, The Netherlands).

A PRRS infection, diagnosed by Elisa serology, seems to play an important role as co-infection, and the vaccination with Porcilis® PRRS (Intervet) modified live vaccine (European DV strain) was decided.

After checking the lack of *App* maternally derived antibodies, the selected vaccination scheme was: PRRS, *M hyo* and *App* at 4 weeks of age, and *App* and *M hyo* at 7 weeks.

During the trial, because of an E. coli outbreak between the first and the second vaccination, this last injection was postponed 2 weeks later, it means at 9 weeks of age.

The trial has been performed on a batch of 272 piglets divided in 2 different groups:

- Experimental group (237 piglets) with simultaneous administrations, at 4 weeks, Porcilis® PRRS was dissolved in Porcilis® *M hyo*, and then mixed in the same vial with Porcilis® APP for an unique injection of 4 ml. At 9 weeks, Porcilis® *M hyo* was mixed in the same vial with Porcilis® APP for an unique injection of 4 ml.

- Control group (35 piglets) with separate administrations, at 4 weeks, 3 injections (3x2ml): Porcilis® PRRS, Porcilis® *M hyo*, Porcilis® APP. At 9 weeks, 2 injections (2x2ml): Porcilis® *M hyo*, Porcilis® APP.

Blood samples were collected 18 days after the second vaccination, centrifuged, and the sera were sent to R&D Laboratory (Intervet International bv, Boxmeer, The Netherlands) to perform Apx I, II, III, IV, OMP Elisa tests and PRRS Elisa tests (IDEXX kit for PRRS).
Results

No adverse systemic and local reactions have been observed in both groups. All the piglets have kept a normal feed intake. At the time we submit this paper, more than 3300 piglets have been vaccinated with this combined vaccination scheme without any adverse reaction.

A total of 66 animals have been sampled (31 in control group, 35 in experimental group). For serological evaluation of App vaccination, unfortunately, some of Elisa Apx IV titers were found positive or doubtful, which probably indicated an early App field infection. So 6 animals from control group and 3 from experimental group were not included in the final interpretation. (Table 1)

The serological evaluation of PRRS vaccination is not easy, because Elisa Idexx kit detected antibodies are not constant (the vaccine works by cellular and humoral immunity), and such antibodies could derived from field infection too. In this study, 53 days after vaccination in both groups, very high S/P ratios are detected, which underlined probably a field infection and not a response to vaccination. We did not dispose of PCR results to confirm a contact with a field strain, but such high S/P ratios, linked with the E. coli outbreak between first and second vaccination could suggest an early PRRS infection. (Table 2)

Table 1: Serological results after App vaccination.

<table>
<thead>
<tr>
<th></th>
<th>Mean titer values: Log 2 (Standard Deviation)</th>
<th>Number of pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Apx</td>
<td>OMP</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Control Group</td>
<td>10.4</td>
<td>11.9</td>
</tr>
<tr>
<td></td>
<td>(1.3)</td>
<td>(1.0)</td>
</tr>
<tr>
<td>Experimental Group</td>
<td>11.4</td>
<td>12.9</td>
</tr>
<tr>
<td></td>
<td>(1.4)</td>
<td>(0.9)</td>
</tr>
</tbody>
</table>

Table 2: Serological results after PRRS vaccination.

<table>
<thead>
<tr>
<th></th>
<th>Number of pigs and mean titer values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S/P &lt; 0.4</td>
</tr>
<tr>
<td></td>
<td>Nb</td>
</tr>
<tr>
<td>Control Group</td>
<td>0</td>
</tr>
<tr>
<td>Experimental Group</td>
<td>2</td>
</tr>
</tbody>
</table>

Discussion and Conclusions

In this field case, before starting the trial, the piglets were vaccinated at 3 different times: at 4 weeks with an M hyo one shot vaccine, at 7 and at 11 weeks with an App vaccine. The tested combined vaccination scheme reduces in the same time the workload with 2 interventions only (and probably improvement of compliance) and it allows to add the PRRS vaccination and to come back to a double shot M hyo vaccination in this PRDC situation.

These preliminary data seem to confirm the possibility to associate these three Intervet vaccines based on the same adjuvant. There was no differences in local or systemic reactions and seroconversion for App between the 2 groups.

References

Materials and methods

This field trial was settled in a farrow-to-finishing farm (700 sows) located in Finistère (West part of Brittany), serologically PRRS and PPV positive. Incoming gilts are purchased from a PRRS-free selection herd. The batches of gilts included in the study were divided, when introduced to farm quarantine, in 2 groups (matched pairs of gilts), according to their vaccination protocol against Ery, PPV and PRRS:
- A control group (A), including gilts vaccinated separately on time with Ery+PPV and PRRS vaccinations.
- An experimental group (B) with both vaccinations (Ery+PPV and PRRS) done simultaneously on the same day.

The used vaccines for this field trial were Porcilis Ery+Parvo (20 ml vials) and Porcilis PRRS (10 doses vials) (Intervet). For the experimental group (B), both vaccines were mixed according a very strict procedure (extra-label use), with 2 ml of the association injected per animal. Injections were performed by intra-muscular way in the neck area with disposable needles.

Blood sampling has been done at 3 successive times: on the first day of the trial (delivery to quarantine), on the day of Porcilis Ery+Parvo booster injection and at the end of the trial, i.e. around 6 weeks after the beginning of the trial.

Experimental scheme was as following:
- D0: inclusion and repartition of gilts; Porcilis PRRS vaccination (group A) or combined Porcilis PRRS/Porcilis Ery+Parvo (group B); blood sampling Nr 1 (BS1).
- D0 + 7 days: Porcilis Ery+Parvo vaccination (group A).
- D0 + 25 days: Porcilis Ery+Parvo booster injection (groups A and B); blood sampling Nr 2 (BS2).
- D0 + 40 days: blood sampling Nr 3 (BS3).

Laboratory analysis done on sera were an Elisa PRRS test (2XR Idexx kit, cut-off level ratio S/P: 0.4) and an Elisa PPV test (LSI kit, cut-off level ratio without vaccination : 0.15) performed in Bio-Chêne Vert laboratory (Chateaubourg, France).

Evaluation of safety has been done on gilts from the first (group A) or unique vaccination (group B) till 7 days after the second (group A) or unique (group B) injection. Then safety concerns were measured for the 7 days following the Porcilis Ery+Parvo booster injection in the 2 groups.

Statistics have been performed with SAS software (ANOVA test).

Results

Two batches of gilts could be included in this trial, i.e. respectively 21 and 23 animals for groups A and B. No systemic nor local reactions were observed on gilts in any group.

Dealing with serologies, 8 animals (3 from group A and 5 from group B) were excluded from the final interpretation, because of a PRRS serological positivity at first sampling (D0) for 6 incoming gilts (but...
PRRSv PCR testing on these animals was fully negative and the selection herd was confirmed negative), and because of an increase of PRRS Elisa titers between BS2 and BS3 with a negative PRRS PCR testing at BS3 for the 2 other gilts. Finally 36 gilts, i.e. 18 animals per group (Table 1), were kept for the final interpretation. There was no statistical difference (p > 0.05) between the 2 groups on all measured serological parameters, at any time of blood sampling. No statistical difference (p > 0.05) was observed between the 2 groups A and B on fertility and prolificacy levels.

Table 1: PRRS and PPV serologies in gilts

<table>
<thead>
<tr>
<th>PRRS/PPV serologies</th>
<th>BS 1</th>
<th>BS 2</th>
<th>BS 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elisa PRRS (S/P)</td>
<td>0.02</td>
<td>0.02</td>
<td>2.32</td>
</tr>
<tr>
<td>Elisa PPV (S/P)</td>
<td>0.35</td>
<td>0.9</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Interpretation of Elisa PPV titers, at BS 3 especially, is quite difficult, because of a field contamination by PPV: 18 gilts among 36 (7 from group A and 11 from group B) have probably been infected by the field Parvovirus.

About PRRS status of the gilts, all the performed PCR tests confirmed the absence of field contamination during the study. PRRS serological titers are representative from a post-vaccinal humoral response with 100 % of «responders» in both groups at BS 2 and BS 3.

Discussion and Conclusions

A first conclusion of the study is the good safety of both vaccination protocols, what is confirmed by previous data (2). Early infection of gilts in quarantine by the field Porcine Parvovirus confirmed the difficulty to estimate on the field a quantitative PPV post-vaccinal immune response. PRRS post-vaccination humoral response is consistent with previous serological data measured in an Italian trial with Porcilis PRRS (2): a maximum of «responders» on both groups (100 %) in 4 weeks after vaccination. Elisa screening is the most common used test to determine success of exposure to a field virus or a modified live vaccine (MLV) virus (1,3). According to Polson (5), groups of «naïve» animals could develop a relatively typical curve of antibody response after first exposure to PRRS virus (infection by field virus or vaccination with a MLV). Field observations (Intervet unpublished data) showed that one injection of Porcilis PRRS vaccine on 6-week old piglets (with maternal derived antibodies) could induce Elisa titers (ratio S/P) from 0.5 to 3 from one month following vaccination. This scale of humoral response seems to be less variable and more narrow for «naïve» animals as the gilts from this study, negative during quarantine, then with Elisa titers (ratio S/P) between 1.5 and 2.5 days after vaccination. To complete data about efficacy of such combination (Ery+PPV and PRRS vaccinations), additional laboratory trials, as challenge experimentations, would be required.

References

3. Idexx Herdcheck PRRS, Monography, Website Idexx Laboratories.
4. Laval A., La problématique de la vaccination chez le porc, Actualités en production porcine, AFMVP Meeting, December 5-6 2002, Alfort, 118-125.
Materials and methods

A. In the first part of this study, the serological response of young piglets with varying levels of MDA to PCV2 vaccination was determined in a number of separate experiments. Young piglets randomly chosen from commercial and high health herds were vaccinated twice with a 2-3 week interval with different doses of the vaccine, up to a full dose. In total 199 piglets were vaccinated, ranging in age between 3 and 46 days of age at the start of the vaccinations (median age: 12 days). These experiments included a total of 106 control animals of similar distribution.

Blood was taken at the time of the first vaccination and again 3 weeks after the booster vaccination. Anti-PCV2 titers were determined using a blocking ELISA where serum antibodies were captured onto immobilised PCV2 capsid protein, followed by incubation with a fixed amount of peroxidise-conjugated anti-PCV2 monoclonal antibody. Titers were expressed as the reciprocal of the dilution giving 50% blocking. The change in serum titer over the 6 week period was correlated to the starting titer.

B. In the second part of the study, 2 animal studies were undertaken where piglets were vaccinated with vaccine formulations containing different doses of antigen, and then challenged intranasally with 4.5 log_{10} TCID_{50} of wild type PCV2 virus. Three weeks after the challenge, virus replication was assessed by Q-PCR of relevant lymphoid organs.

Results

A. Starting titers of the piglets ranged between <1.6 log_{2} (n=2) and 15.4 log_{2}, 25% was lower than 6.4 and 25% was higher than 10.3 log_{2}. As expected, the decline in serum titers of the control animals during the 6 week test period was not dependent on the starting titer (data not shown). In a few cases, with MDA levels on or below 6 log_{2}, an antibody increase was actually observed, presumably indicating a field infection in these animals.

On the other hand, in vaccinated animals, the change in antibody titer over time was clearly related to the level of MDA at the time of first vaccination. MDA levels above appr. 7 log_{2} prevented a seroresponse following vaccination with low-dose vaccine. Vaccine containing a high dose of antigen broke through MDA levels of appr. 10 log_{2} (Fig.1, see also section B).
B. In the first challenge experiment, 8-12 day old piglets with MDA titers of $9.2 \pm 1.6 \log_2$ were vaccinated with formulations containing 1/16 or 1/64 dose of antigen. Control animals were not vaccinated. Challenge took place 2 weeks after the booster vaccination, at 5 - 5.5 weeks of age. At that time, only 2/10 and 0/9 of the animals vaccinated with respectively 1/16x and 1/64x vaccine had seroresponded. At 3 weeks post challenge, Q-PCR on tonsil and mesenteric lymph nodes indicated that compared to the unvaccinated animals, the animals treated with 1/16 and 1/64 dose of antigen had a 6-16 fold reduction in virus load (Fig.2).

In the second challenge experiment, 3-5 day old piglets with MDA titers of $7.6 \pm 1.7 \log_2$ were vaccinated with a full dose of the vaccine. At 2.5 weeks after the booster, at 6 weeks of age, the animals were challenged as before. At the time of challenge, 7/9 of the vaccinates had seroresponded. At 3 weeks post challenge, the virus load in tonsil and mesenteric lymph nodes was respectively 16,000 and 8,000-fold reduced in the vaccinated animals (Fig.3). Tissues from 6/10 control animals contained levels of viral DNA indicative of PMWS, compared to none of the vaccinates.

**Discussion and Conclusions**

From these results it is clear that PCV2 vaccination in the face of maternally derived antibodies is possible, but that the level of antibodies at the time of vaccination greatly influences the effectiveness of the vaccination. A full dose of the vaccine was necessary to overcome the highest levels of MDA.

The challenge experiments confirmed these findings, showing that a significant reduction of PCV2 replication could be achieved, preventing virus levels known to be associated with PCVD. This suggests that PCVD can be prevented by a full dose of PCV vaccine, even in MDA+ piglets challenge infected at 5-6 weeks of age.

**References**

FIELD TRIALS TO ASSESS THE PERFORMANCE OF A CONDITIONALLY LICENSED PCV2 VACCINE IN CANADA

F. de Grau¹; B.Thacker²; C.Francisco²; W.Wilson²; Rich Schlueter²; A.Eggen³
¹Intervet Canada, Whitby, Ontario; ²Intervet Inc, Millsboro, Delaware; ³Intervet International Boxmeer Holland.

Introduction and Objectives
A previous study involving 35,000 vaccinated pigs demonstrated promising results in the field for controlling the PCV2 outbreak in Canada using a conditionally licensed PCV2 vaccine developed by Intervet Inc.¹ Our objective was to gather information on clinical trials performed on commercial swine operations that used Intervet PCV2 vaccine, during the 2006 PCV2 outbreak in Ontario and Quebec.

Materials and methods
The data was kindly provided by two Ontario swine veterinarians and one veterinarian from Québec. Close out information from 12,700 pigs from Ontario and 1,520 pigs from Quebec was provided to Intervet. The PCV2 vaccine was purchased from Intervet by the veterinarians and Intervet played no role in the design and conduct of these studies.

The disease status of the operations ranged from: high health status, free of Mycoplasma hyopneumoniae, and Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), to herds where many swine pathogens were present including Mycoplasma hyopneumoniae, PRRSV, and swine influenza virus. The three veterinarians performed clinical trials comparing vaccinated (Vx) and non-vaccinated (Non-Vx) pigs within the same farm, to assess the performance of Intervet’s PCV2 vaccine.

Historical mortality by group ranged from 6.85 to 16.00 %.

Results
In a large clinical trial, a production company in Ontario vaccinated 4,300 pigs and compared their performance to 5,780 Non-Vx pigs within the same system. The farms had been suffering from PCV2 prior to the availability of the vaccine. Historical mortality by group ranged from 6.85 to 16.00 %. On PRRSV and mycoplasma negative farms, mortality for Non-Vx pigs was 5.83 % on average compared to 0.77% for Vx pigs. Average daily gain for Vx pigs was 0.840 kg while Non-Vx pigs had an ADG of 0.720 kg, an improvement of 16.7 %. Days to market (DM) were significantly lower for Vx pigs compared to Non-Vx (109 vs. 121 respectively). Feed conversion (FC) was significantly better as well; 2.59 for Vx pigs vs. 2.73 for Non-Vx pigs.
Within the same company but in a PRRSV negative and mycoplasma positive production system where the historical mortality was 16.08 %, Vx pigs had a mortality of 0.60 % while Non-Vx pigs had a mortality of 4.60 %. ADG was 0.870 kg vs. 0.660 kg; DM, 106 vs.113 days and FC, 2.59 vs. 3.00 for Vx vs. Non-Vx pigs, respectively. In addition, the culling rate due to light weight was also greater for Non-Vx pigs compared to Vx pigs; 5.7 % vs. 1.0 %, respectively.

On another Ontario system suffering from PRRSV and mycoplasma, the performance of 1,294 Vx pigs vs. 1,300 Non-Vx pigs, respectively, was: mortality, 2.00 % vs. 7.60 %; ADG, 0.913 kg vs. 0.793 kg; FC 2.63 vs. 2.98; and culling rate, 1.1 % vs. 4.2 %.

In Quebec, a field trial was performed to assess PCV2 vaccination performance in a 1,525, PRRSV and mycoplasma positive, grower-finisher barn.; 1,050 pigs were vaccinated and 475 were kept as Non-Vx controls. Mortality for Non-Vx pigs was 9.00% versus 1.05% for Vx pigs. The rate of light weights and culls for Non-VX pigs was 2.8 % compared to 2.4 % for Vx pigs.

The vaccine was effective regardless of the health statuses within the herds. Improvement in all production performance parameters was observed in Vx pigs compared to Non-Vx pigs in all herds.

Discussion and Conclusions

Since the emergence of PCV associated disease in Canada, producers and veterinarians have attempted to control the outbreak using a number of approaches prior to vaccine being available2. Publication of field data performance and field trials has not been available until now3.

The vaccine was effective regardless of the health statuses within the herds. Improvement in all production performance parameters was observed in Vx pigs compared to Non-Vx pigs in all herds.

Acknowledgements

The authors would like to thank all the volunteer producers and veterinarians involved on this study.

References

**Materials and methods**

The trial was designed as a multi-centred, randomized and conducted in farms in Ontario, Quebec and Manitoba. Five veterinary practices agreed to participate voluntarily on this project. Twenty-one farms were selected by the practitioners. PCV2 was known to be present on these farms and clinical disease associated with this virus had been demonstrated to be a serious problem. In total, 35,000 pigs from 2 farms were included in the study from April 2006 until market weight was achieved. The pigs were vaccinated initially within 3–5 weeks of age and booster 3 weeks later. The animals were monitored closely from day 1 until 6 days after each vaccination. Injection site reactions, feed intake, and general health were used as parameters to assess the safety of the vaccine. Mortality reduction and close out information was used to assess efficacy compared to non vaccinated animals within the same production system.

**Introduction and Objectives**

Starting in fall of 2004 an outbreak of Porcine Circovirus spread through Ontario and the Quebec provinces. Mortalities ranging from 8 to 50% had been experienced since then due to the virus. There has been a decline in market hog production in of approximately 2% in Ontario alone. The disease once confined to hogs 6 to 10 weeks of age is now showing up in hogs aged 10 to 15 weeks. Many producers have been debating whether to remain in pig production in light of the severe economic losses associated with this outbreak. To date estimated losses have been 100,000 pigs in Ontario and 270,000 in Quebec. The Canadian Pork Council approached the animal health companies for help. Intervet was one those companies that responded to this industry need. Intervet Canada conducted a trial to assess the efficacy and safety of a Porcine Circovirus Type 2 vaccine, developed by Intervet Inc. under field conditions in Canada.

**FIELD PERFORMANCE OF A CONDITIONALLY LICENSED VACCINE: CANADIAN EXPERIENCE**

F. de Grau1; B.Thacker2; C.Francisco2; W.Wilson2; Rich Schlueter2; A.Eggen3

1Intervet Canada, Whitby, Ontario; 2Intervet Inc, Millsboro, Delaware; 3Intervet International Boxmeer Holland.

Intervet Canada conducted a trial to assess the efficacy and safety of a Porcine Circovirus Type 2 vaccine, developed by Intervet Inc. under field conditions in Canada.
Results and discussion

In total, 35,653 pigs received the first vaccination at 4 weeks of age (SD± 2) and 35,108 pigs received a booster dose at 7 weeks of age (SD± 2). Five hundred forty-four vaccinated piglets died before the second injection (1.52 %) due to non PCV2 related causes. One piglet died due to an anaphylactic reaction related to vaccination (0.002 %). A total of 5,900 piglets (16 %) had local reactions to the vaccine, swellings ranging from the size of a pea to an egg size. All investigated swellings were cool to the touch were not accompanied by fever or elevated body temperature and began to reside quickly and disappeared in 2-3 weeks with no side affects. These swellings may be farm related as they have only been reported in significant numbers from few sites. No pigs were treated due to swellings or local reactions and no carcass condemnations were associated with this event. Vaccinated pig mortality was lowered 77.5 %, when compared to non-vaccinated pigs within the same groups. The mortality for vaccinated pigs was on average 2.1 %.

Farms vaccinating pigs initially after 5 weeks of age experienced higher grower-finisher mortality (4 %) compared with 1.54 % on farms vaccinating initially within 3 weeks of age. It is believed that some of the groups vaccinated initially after 5 weeks of age were already viremic after both doses were administered.

However, mortality was still reduced by 75 % versus controls for farms vaccinating initially at 7 weeks as compared to 78 % reduction of mortality on farms vaccinating initially at three weeks of age. Mortality seen in the grower finisher period from pigs vaccinated with Porcine Circovirus Type 2 vaccine, (Intervet Inc.) is comparable to “normal” production parameters (2% maximum)³. Producers and veterinarians are calling the success of vaccination a miracle⁴.

Acknowledgements

The authors would like to thank all the volunteer producers and veterinarians involved on this study.

References

1. Agri-food Canada report March 2006 http://atssea.agr.gc.ca/can/4028000_e.htm
2. Procter K. PMWS our most significant and costly health challenge. Better Pork April 2006

Mortality was reduced by 75 %.

Producers and veterinarians are calling the success of Porcine Circovirus Type 2 vaccine a miracle.
IMPACT OF PORCINE CIRCOVIRUS ASSOCIATED DISEASE (PCVAD) ON FINISHERS PERFORMANCE COMPARING TWO DIFFERENT PRODUCTION SYSTEMS IN MEXICO

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¹Intervet-México, Huixquilucan, México; ²Benemérita Universidad Autónoma de Puebla, Tecamachalco, Puebla, México; ³Intervet-International, Boxmeer, The Netherlands.

Introduction and Objectives

Porcine Circovirus type 2 (PCV2) is associated with two syndromes Postweaning Multisystemic Wasting Syndrome (PMWS) and Porcine Dermatitis and Nephropathy Syndrome (PDNS), and also with reproductive failure. Recently in the USA the general name PCVAD was suggested. The objective of this trial was to compare the effects of PCVAD in two different production systems.

Materials and methods

Two high health, PRRS-free, Mexican farms, were chosen: one of 600 sows utilizing a one-site system, and the other of 750 sows, managed on three segregated sites.

For the year before, and the year following the first appearance of clinical signs, each weekly batch of pigs produced was evaluated with respect to mortality, average daily gain (ADG) and feed conversion. Statistical analysis was performed using the ANOVA test. Histological examination of lymphoid tissue from pigs showing clinical signs of PMWS and PDNS identified intra-cytoplasmic inclusion bodies, lymphocyte depletion, multinucleated giant cells and infiltration of histiocytes. Diagnosis was further confirmed by immunohistochemistry (IHC).

Results

There were noticeable differences in mortality rate, ADG and feed conversion between the two production systems.

Table 1: Weaner productivity (21 – 70 days of age).

<table>
<thead>
<tr>
<th>System</th>
<th>One site</th>
<th>Segregated 3-site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µ1 ± α</td>
<td>µ1 ± α</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>4.46 ± 1.61</td>
<td>2.18 ± 1.32</td>
</tr>
<tr>
<td>ADG (kg.)</td>
<td>0.474 ± 0.03</td>
<td>0.485 ± 0.02</td>
</tr>
<tr>
<td>Feed conversion</td>
<td>1.46 ± 0.15</td>
<td>1.42 ± 0.09</td>
</tr>
</tbody>
</table>

Table 2: Finisher productivity (71 – 150 days of age).

<table>
<thead>
<tr>
<th>System</th>
<th>One site</th>
<th>Segregated 3-site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µ1 ± α</td>
<td>µ1 ± α</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>8.66 ± 3.29</td>
<td>4.38 ± 1.31</td>
</tr>
<tr>
<td>ADG (kg.)</td>
<td>0.691 ± 0.04</td>
<td>0.734 ± 0.05</td>
</tr>
<tr>
<td>Feed conversion</td>
<td>3.46 ± 0.39</td>
<td>2.88 ± 0.18</td>
</tr>
</tbody>
</table>

µ1 = Population mean
± α = Standard deviation
* = p < 0.05
In one-site production systems, the PCVAD impact is greater than in segregated-three site production systems.

The mortality rate in the weaners during the PCV2 outbreak increased by 104.6% and ADG was reduced by 2.86%.

Discussion and Conclusions

In reference to the previous year productivity parameters without clinical signs attributable to PCV2, the mortality rate in the weaners during the PCV2 outbreak increased by 104.6% and ADG was reduced by 2.86%. Huerta (2004) reports a weaner mortality rate of 4.05% in Mexican intensive pork industry, the rate in the one-site unit was 10.12% more. The segregated system had lower mortality than the one-site system (p <0.05), and ADG also was significantly different. Feed conversion was within the normal range, slightly greater into on the one-site farm due to the high mortality (Table 1).

There was 100% increase in mortality rate in the finishers of both systems, ADG and feed conversion were also negatively affected by the PCVAD. The one-site system was more severely affected than the three-sites system (Tables 1 & 2). Post mortem examinations revealed 70% deaths due to PMWS and 10% due to PDNS.

From these data we conclude:

• In one-site production systems, the PCVAD impact is greater than in segregated-three site production systems.
• The most commonly diagnosed clinical syndrome was PMWS followed by PDNS.

According with the author’s experience on Mexican farms, the PCVAD clinical signs are more obvious in PRRSV-free Mexican herds with a high health status.

References

ASSOCIATION OF GLAESSER´S DISEASE WITH PRRSV AND PCV2 INFECTION

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²Institute for Pathology of the Ludwig-Maximilians-University, Munich, Germany

Introduction and Objectives

The influence of an infection with the Porcine Respiratory and Reproductive Syndrome Virus (PRRSV) on the development of the Glaesser´s disease was investigated in field samples. Segales et al. (1999) found no influence of a previous infection with PRRSV on the occurrence of Haemophilus (H.) parasuis infections. In contrast Solano et al. (1997) described an influence of double infections on the mortality of the piglets. They also found a decrease in the ability of porcine alveolar macrophages to kill bacteria after a PRRSV infection (Solano et al., 1998).

Materials and methods

A total of 56 pigs from 20 different farms were included in this study. Clinical evaluation concentrated particularly on the respiratory system, joints and central nervous system. After the clinical evaluation the pigs were euthanased and examined post-mortem. At necropsy dry swabs were taken (Palzer et al., 2006). These were analysed within 2 days by PCR on H. parasuis specific genome sections (Landeslabor Schleswig-Holstein). At necropsy samples of the lung and the lymph nodes were taken and analysed by a PCR for PRRSV (EU-Type) and PCV2 specific genome sections. A fibrinous inflammation of pericardium, pleura and/or peritoneum was defined as fibrinous serositis. Statistical significance was calculated using the chi-square test.

Results

A total of 25 (45%) pigs were positive for H. parasuis on PCR. The clinical examinations showed first evidence of infection. A total of 14 (25%) pigs were positive for PRRSV (EU-Type) on the PCR. The association between H. parasuis on PCR and PRRSV on PCR was significant (p=0.02) (Table 1). There was no significant association between a positive H. parasuis PCR and a positive PCV2 PCR (p=0.97).

<table>
<thead>
<tr>
<th></th>
<th>PRRSV PCR positive</th>
<th>PRRSV PCR negative</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. parasuis PCR positive</td>
<td>10</td>
<td>15</td>
<td>25 (45 %)</td>
</tr>
<tr>
<td>H. parasuis PCR negative</td>
<td>4</td>
<td>27</td>
<td>31 (55 %)</td>
</tr>
</tbody>
</table>

There was no significant association between a positive H. parasuis PCR and a positive PCV2 PCR (Table 2).

Table 1: Association between Haemophilus parasuis PCR on serosal swabs and PRRSV PCR of lung and lymph node samples

Table 2: Association between Haemophilus parasuis PCR on serosal swabs and PCV2 PCR of lung and lymph node samples
There was a positive association between the detection of H. parasuis in PCR and fibrous serositis (p=0.007).

**Discussion and Conclusions**

The detection of H. parasuis in dry swabs of the serosal surfaces correlates with the finding of a serositis in the necropsy.

In the present study, there was a significant association between the detection of H. parasuis in collective swabs of the pericardium, pleura and peritoneum and PRRSV (EU-Type) specific genom fragments in samples of the lung and lymph nodes. The interaction of PRRSV infections and bacterial endotoxins was investigated by van Gucht et al. (2003) who showed a synergism between PRRSV and the administration of bacterial lipopolysaccharides in the development of respiratory diseases. Because pigs are infected with H. parasuis via aerogenic route, it is possible that an infection occurs more often in pigs which are PRRSV positive. This explains that PRRSV positive animals develop often Glaesser’s disease significantly more often. The results of the presented study indicate that there is no influence of a PCV2 infection on the development of Glaesser’s disease or vice versa, Glaesser’s disease has no influence an infection with PCV2.

There was no positive association in the detection of PRRSV and PCV2. Nevertheless further investigation has do be done, because a tendency can be seen.

**Acknowledgements**

This study was supported by Intervet, Germany

**References**

Introduction and Objectives

Swine influenza virus (SIV) has been present in Mexico’s swine industry for a few years. In the beginning it was associated with only mild signs, so diagnosis was mainly by serology. The present trial was initiated to find out whether a commercial vaccine would be able to stabilize field antibodies.

Materials and methods

On a Mexican commercial S1 farm all sows (4,800) were vaccinated twice (2ml per dose, 3 weeks apart) with End-FLUence®2 (Intervet). Serological test were carried out on 30 sows (5 per parity, from 1st to 6th parity) both before (Haemagglutination Inhibition test), and one month after (ELISA Idexx®) vaccination against SIV H1N1 subtype, the prevalent subtype on the farm.

Results

Table 1: Average values (titre or S/P) per parity before & after vaccination and standard deviation after vaccination. Standard deviation and coefficient of variation of average values before and after vaccination.

<table>
<thead>
<tr>
<th>TIME</th>
<th>BEFORE</th>
<th>AFTER</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEST</td>
<td>HI</td>
<td>ELISA</td>
</tr>
<tr>
<td>PARITY</td>
<td>TITRE</td>
<td>S/P</td>
</tr>
<tr>
<td>1</td>
<td>80</td>
<td>1.192</td>
</tr>
<tr>
<td>2</td>
<td>912</td>
<td>1.308</td>
</tr>
<tr>
<td>3</td>
<td>960</td>
<td>1.279</td>
</tr>
<tr>
<td>4</td>
<td>1280</td>
<td>1.496</td>
</tr>
<tr>
<td>5</td>
<td>1025</td>
<td>1.408</td>
</tr>
<tr>
<td>6</td>
<td>1024</td>
<td>1.422</td>
</tr>
<tr>
<td>AVERAGE</td>
<td>880.167</td>
<td>1.351</td>
</tr>
<tr>
<td>S. D.</td>
<td>412.113</td>
<td>0.111</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>46.82</td>
<td>8.22</td>
</tr>
<tr>
<td>Cut-off</td>
<td>80</td>
<td>0.4</td>
</tr>
</tbody>
</table>

The present trial was initiated to find out whether a commercial vaccine would be able to stabilize field antibodies.
Discussion and Conclusions

Different tests were employed before and after vaccination, so while the effect was well enough demonstrated, the results could not be subjected to statistical analysis. Instead, average titres and values were plotted to show trends only. The trend curves, shown in the graphs (Figure 1 & 2), clearly demonstrate the stability achieved following vaccination.

Figure 1 shows how the 1st party sows were naïve at the time of their introduction to the herd (average titre 1:80, the cut-off titre) to SIV H1N1 subtype, and how following their introduction, they became increasingly infected, as demonstrated by the rising titres from 1:912 and 1:960 (parities 2 & 3) to 1:1280 (highest) in parity 4 sows, and 1:1025 and 1:1024 in the oldest sows.

The curve in Figure 2 shows how, one month after vaccination, all S/P average values (from all parities) had achieved excellent stabilization, included 1st parity sows which had been naïve beforehand (Figure 1). This is supported by the low standard deviations shown in Table 1.

On the basis of this field study it is possible to conclude that a herd-wide sow vaccination programme is effective in achieving rapid and appropriate antibody stabilization.

Acknowledgements

Drs. Hernandez Daniel, Deportes José Miguel & Ochoa Víctor from Granjas Carroll de México; Ramírez Humberto & Fernández Rocío from Intervet – México.

References


Materials and methods

On a commercial one-site farm (600 sows) with unknown maternal antibodies levels. One hundred and fifty pigs were divided in three groups according their age of 5, 6 and 7 weeks, 50 animals per age-group were vaccinated twice (3 weeks interval) with a commercial bivalent vaccine (End-FLUence2; Intervet). From each group 10% (always the same 5 pigs) were weekly blood taken in total 8 times. All the serum samples were stored frozen until their serological evaluation by ELISA (Idexx®) or HI tests was made. All activities per group started and finished the same day.

Results

The weekly average results of both the ELISA and the HI tests per age at 1st dose are shown in Table 1. To compare both tests graphs (Figures 1 and 2) are shown to see the curve trends.

Table 1: Average weekly values (HI titre or ELISA S/P) per Group-Age at 1st vaccination and average, standard deviation and Coefficient of variation per Group and test.

<table>
<thead>
<tr>
<th>GROUP (WEEKS OF AGE AT 1ST VACCINATION)</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEST</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st Vaccination</td>
<td>HI</td>
<td>ELISA</td>
<td>HI</td>
</tr>
<tr>
<td>1 WEEK AFTER</td>
<td>100</td>
<td>1.202</td>
<td>64</td>
</tr>
<tr>
<td>2 WEEKS AFTER</td>
<td>96</td>
<td>0.998</td>
<td>44</td>
</tr>
<tr>
<td>3 WEEKS AFTER &amp; 2nd Vaccination</td>
<td>64</td>
<td>0.700</td>
<td>36</td>
</tr>
<tr>
<td>1 WEEK AFTER</td>
<td>136b</td>
<td>1.189b</td>
<td>96a</td>
</tr>
<tr>
<td>2 WEEKS AFTER</td>
<td>136b</td>
<td>1.189b</td>
<td>96a</td>
</tr>
<tr>
<td>3 WEEKS AFTER</td>
<td>80b</td>
<td>0.834a</td>
<td>160a</td>
</tr>
<tr>
<td>4 WEEKS AFTER</td>
<td>72a</td>
<td>1.033a</td>
<td>80a</td>
</tr>
<tr>
<td>AVERAGE</td>
<td>80</td>
<td>0.971</td>
<td>76</td>
</tr>
<tr>
<td>S. D.</td>
<td>31</td>
<td>0.22</td>
<td>40</td>
</tr>
<tr>
<td>C. of variation</td>
<td>38</td>
<td>22.3</td>
<td>53</td>
</tr>
</tbody>
</table>

Values with different letter are different (P< 0.05; T student test) respect to 2nd vaccination day titre or S/P
Figure 1: HI weekly average titre per Group.

Figure 2: ELISA weekly average S/P values per Group.

Discussion and Conclusions

The objective of this field study was to know the antibody stimulation to attenuated Swine influenza virus (SIV) - H3N2 subtype.

It is possible to vaccinate in the presence of moderate - high maternal derived antibodies level resulting in a normal serological response.

The MDA at 1st vaccination day not show the expected standard according with the age; Groups 5 and 7 had highest similar titres by HI test, Group 6 had the lowest values on both tests. At the 2nd vaccination day all the groups showed similar titres by HI test, but not by ELISA test showing clearly reduced S/P values compared to values at 1st vaccination day. In the HI test the Groups 5 and 6 showed peaks after the 2nd vaccination; at week 2 and at the week 3 respectively, the response of the Group 7 was plateau shaped but with lower HI titres. In the ELISA test after the 2nd vaccination Group 5 showed a plateau curve response but with a little decline at week 3, the Group 6 had 2 peaks at the weeks 1 and 3, and the Group 7 showed an increase curve trend but with lower S/P values. In both tests the last point (4 weeks after the 2nd vaccination day) was nearly the same in all 3 Groups.

These results show that it is possible to vaccinate in the presence of moderate - high maternal derived antibodies level resulting in a normal serological response.
Materials and methods
On a commercial one-site farm (600 sows) with unknown maternal antibodies levels. One hundred and fifty pigs were divided in three groups according their age of 5, 6 and 7 weeks, 50 animals per age-group were vaccinated twice (3 weeks interval) with a commercial bivalent vaccine (End-FLUence2; Intervet). From each group 0% (always the same 5 pigs) were weekly blood taken in total 8 times. All the serum samples were stored frozen until their serological evaluation by ELISA (Idexx®) or HI tests was made. All activities per group started and finished the same day.

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<table>
<thead>
<tr>
<th>GROUP (WEEKS OF AGE AT 1ST VACCINATION)</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEST</td>
<td>HI</td>
<td>ELISA</td>
<td>HI</td>
</tr>
<tr>
<td>1st Vaccination</td>
<td>52</td>
<td>0.765</td>
<td>40</td>
</tr>
<tr>
<td>1 WEEK AFTER</td>
<td>44</td>
<td>0.772</td>
<td>32</td>
</tr>
<tr>
<td>2 WEEKS AFTER</td>
<td>26</td>
<td>0.577</td>
<td>16</td>
</tr>
<tr>
<td>3 WEEKS AFTER &amp; 2nd Vaccination</td>
<td>22a</td>
<td>0.723a</td>
<td>26a</td>
</tr>
<tr>
<td>1 WEEK AFTER</td>
<td>136b</td>
<td>0.992a</td>
<td>88b</td>
</tr>
<tr>
<td>2 WEEKS AFTER</td>
<td>152b</td>
<td>1.118b</td>
<td>128b</td>
</tr>
<tr>
<td>3 WEEKS AFTER</td>
<td>136b</td>
<td>0.661a</td>
<td>84b</td>
</tr>
<tr>
<td>4 WEEKS AFTER</td>
<td>88b</td>
<td>0.975a</td>
<td>56b</td>
</tr>
<tr>
<td>AVERAGE</td>
<td>82</td>
<td>0.823</td>
<td>59</td>
</tr>
<tr>
<td>S. D.</td>
<td>53</td>
<td>0.19</td>
<td>38</td>
</tr>
<tr>
<td>C. of variation</td>
<td>65</td>
<td>22.5</td>
<td>65</td>
</tr>
</tbody>
</table>

Values with different letter are different (P< 0.05; T student test) respect to 2nd vaccination day titre or S/P.
Discussion and Conclusions

Both tests show at the beginning of the trial (1st vaccination day) a similar MDA values; Group 7 was the highest on MDA. HI titres between groups were similar until the 2nd vaccination, but not so with the ELISA test. Group 5 had a higher HI plateau level than Group 6, in the ELISA test their antibody response was similar but went down at week 3 after the 2nd vaccination. Group 6 showed an antibody plateau response on both tests after the 2nd vaccination, in the HI test the lowest but similar as the other groups in the ELISA test. Group 7 had in the HI test the only peak, and the highest titre, 2 weeks after the 2nd vaccination, but by ELISA their response after the 2nd vaccination was plateau shaped from week 1 to 4, a similar curve as those of Group 6. ELISA test had a better Standard deviation and Coefficient of variation result than HI test at the analysis of all the average values per group (Table 1).

Both tests show that even in the face of moderate - high maternal derived antibodies a good sero-response can be expected.