Porcine reproductive and respiratory syndrome (PRRS). Understanding infection dynamics through serum profiles

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Introduction

Porcine Reproductive Respiratory Syndrome (PRRS) was a problem, is a problem and will be a problem in pig farming. The aim of the vet who is facing this disease is to get a PRRS stabilize population of animals. PRRSV serologic screening results are essential to understand the PRRSV epidemiology in the farm, which is necessary to take the proper decisions in each PRRSV infection scenario. So, in the pig veterinary practice, transversal serum profiles are usually implemented to take most proper decisions in order to control PRRSV.

Materials and Methods

200 transversal serum profiles from 200 different farms located in Spain, Hungary, Poland, Czech Republic, Slovakia, Italy and Denmark were analysed. Each serum profile was made from serum samples collected from different pig groups (gilts, sows, piglets and fattening pigs). The sows were grouped together by age (by parities), they were sampled from day of gestation 70 to 100. Piglets and fattening pigs were sampled by weeks of age (4, 7, 10, 13, 16, 19, 21 and 24). The sample was blood sera and the amount of samples per group depended on the size of the farm and the estimated infection prevalence. The average number of serum samples per group was 7. Serology analysis was made with ELISA (CIVTESTsuis PRRS/ES Laboratorios Hipra, Amer, España) following the manufacturer instructions. From each monitored farm was noted the productive data, the vaccination program and the clinic situation.

Results

After testing and analyzing 200 farms, we could determine 3 clear PRRSV infection patterns which let us to define 3 different PRRSV epidemiologic status: 1. Clinically not stable and serologically not stable, (42 farms out of 200) (Graphic 1); 2. Clinically stable and serologically not stable, (112 out of 200) (Graphic 2); 3. Clinically stable and serologically stable, (36 farms out of 200) (Graphic 3). 10 farms could not be classified in any of these 3 patterns.

Discussion

Samples results from pigs at 7 weeks of age became essential information. Passive immunity does not reach with high IRPC levels to 7 weeks of age. If we observe high antibody titters at this age we can suspect that the piglets were already vireamic at weaning and for this reason, we can suspect that. So sows were probably farrowing infected PRRSV piglets. In farms where the piglets were vaccinated, we could not determine any pattern, because vaccination serology response could be confused with a serology positive result due to a wild PRRSV infection. However PRRSV sow vaccination does not interfere with this classification, since we observe that the serology response induced by vaccination does not result in IRPC values higher than 100. Recent wild virus infection induces IRPC values higher than 100. We observed that the evolution of PRRSV infection in farms followed these 3 statuses, evolving from one status to the other within 6 to 12 months period. We do not observe differences among the farm status from different countries. We observed that most of the farms that implemented at least vaccination in sows belonged to the second PRRSV infection pattern (Graphic 2). To sum up, according to this classification, swine vet specialists can monitor and take their proper decisions to control PRRSV.

References

Porcine reproductive and respiratory syndrome (PRRS) on a large pig farm in Slovenia

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Introduction
PRRS is an economically significant viral disease of swine (1). Slovenia was PRRS free country and later on country with low seroprevalence to PRRS in small pig farms (2). In last years seroprevalence to PRRS increased on small farms and also large pig farms became positive. Vaccination after PRRS outbreak is used control measure (3). In 2008 in one of the large one site pig farm production results and increased mortality leaded to suspision of PRRS. PRRS was confirmed in May 2008 with testing of 152 pig with ELISA, from which 112 were positive to PRRS. Farm started with control measures.

Materials and Methods

Vaccination programme
• June 2008 all sows on the farm were vaccinated with inactivated vaccine in three weeks.
• August 2008 vaccination with live vaccine. Sows were vaccinated 6 th day after farrowing and 60 th day of pregnancy.
• From July to November piglets were vaccinated with live vaccine at beginig all and after at age of 14 days.
• December 2008 piglets were vaccinated at age of 24 days.
• From may 2009 till October piglets were not vaccinated.
• From August 2009 in sows at 60 th day of gestation the inactivated vaccine was used.
• October 2009 all sows were vaccinated with live vaccine in 2 days and all piglets were vaccinated at weaning.
• In January 2010 all sows were vaccinated with live vaccine.

Serological control
In year 2008 were tested 101 samples from June to December, and in 2009 562 samples with IDEXX PRRS ELISA.

Selected production Results
Results of 2007, 2008 and 2009 were compared (abortions, farrowing rate, mating rate, weaned piglets per litter, preweaning mortality, postweaning mortality).

Results
In 2008 only 48 samples (47,5%) were seropositive. In 2009 537 (95,5%) sera reacted positive.

Table 1: Comparison of production Results

<table>
<thead>
<tr>
<th></th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abortions/month</td>
<td>14,17</td>
<td>43,83</td>
<td>66,25</td>
</tr>
<tr>
<td>Farrowing rate (%)</td>
<td>83,74</td>
<td>80,11</td>
<td>69,78</td>
</tr>
<tr>
<td>Mating rate (%)</td>
<td>84,81</td>
<td>83,31</td>
<td>74,31</td>
</tr>
<tr>
<td>Weaned/litter</td>
<td>9,68</td>
<td>8,94</td>
<td>8,58</td>
</tr>
<tr>
<td>Preweaning mortality (%)</td>
<td>12,10</td>
<td>8,55</td>
<td>20,20</td>
</tr>
<tr>
<td>Postweaning mortality (%)</td>
<td>4,95</td>
<td>5,58</td>
<td>7,20</td>
</tr>
</tbody>
</table>

Average number of abortions per month in year 2008 from January to October was 14,8 but increased in November to 153 and in December to 212. In 2009 average number of abortions from January to October was 59,7 monthly, but in November 102 and in December 23.

Discussion
PRRS reached culmination in November in December 2008 in spite of used programme of vaccination. Success of vaccination in 2009 was not satifactory. In October 2009 the vaccination scheme was changed fundamantaly. With vaccination of all breeding sows in short interval stabilization of herd was expected. Preliminary results shows that number of abortions decreased significantly and number of seropositive animals is improving. Results in January 2010 indicates that vaccine did not achieve long lasting immunity and PRRS virus still circulates in breeding herd and that repeat vaccination of all sows is appropriate measure to improve production results.

References
Impact of the Porcine Reproductive and Respiratory Syndrome (PRRS) on reproductive performance in Austrian sow herds

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Introduction
Since the first appearance of porcine reproductive and respiratory syndrome (PRRS) in 1987 in the USA and in the early 1990’s in Europe PRRSV has become endemic in most pig producing countries and causes high economic losses (1). PRRSV causes a persistent infection and virus excretion can be detected over several months post infection (2). However, data to substantiate the role of PRRSV on reproductive performance of sows in Austrian pig herds were not available. Due to the different structure of the farms, the data obtained from other countries with much higher concentrated pig herds and higher numbers of animals per herd are not directly comparable to the situation in Austria. So the aim of the study was to evaluate the effect of PRRSV on the reproductive performance of Austrian sow herds.

Materials and Methods
140 Styrian (South-East Austria) production herds were randomly selected. The mean number of sows per herd was 74 animals. The main inclusion criteria were that the farmers had to use the SPon Web soft-ware for reproductive management and to be a member of the Styrian animal health service. On each farm 14 random blood samples were collected. These samples comprised 4 sows, 4 gilts, 4 growers, and 2 boars, if available. All samples were analysed by the IDEXX Herdcheck® PRRS Antibody (Ab) test kit.

Then, the farms were divided into three groups: PRRSV-Ab-positive (at least one animal seropositive, no history of vaccination, n=66), PRRSV-Ab-negative (n=60) or vaccinated (EU-strain MLV vaccine, n=14). Following, reproductive performance data were compared between these three groups.

Results
No significant difference in the number of live born piglets per sow and year could be found between the PRRSV-Ab-negative (median=27.4) and the PRRSV-Ab-positive (median=27.0) group. In contrast the vaccinated herds exhibited a significantly (p<0.05) worse productivity with a median of 25.2 live born piglets. However, the PRRSV-Ab-positive herds showed a trend towards greater losses in the suckling period (median=14.6) than PRRSV-Ab-negative (median=13.2) and vaccinated herds (median=11.4).

Discussion
This study shows that the results from the USA are not directly comparable to the situation in Europe, especially to the one in Austria with its different structure of swine herds. Our data show, that PRRSV-Ab-positive and PRRSV-Ab-negative herds can obtain nearly the same reproductive performance, given PRRSV infection is stable. Interestingly, the number of live born piglets was significantly lower in vaccinated herds. There are several possible explanations for this finding, for example the vaccine does not cover all relevant strains or vaccination regimens were not optimal. However, vaccinated herds achieved better results during the suckling period, which could be a consequence of the initially smaller litter sizes in vaccinated sow herds.

References
Comparison of temporal variation of blood parameters between PRRS naive “5 kg” gilts and PRRSv infected resident piglet population commingled in a nursery

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Introduction
As a method of gilt acclimatization to porcine reproductive and respiratory syndrome virus (PRRSv), many swine producers commingle naive “5 kg” replacement gilts (NG) with their PRRS positive resident nursery pigs. Surprisingly, the NG often perform better than the resident piglets (RP). The objective of this study was to describe and compare temporal variation of hematologic, serologic and viremic characteristics between NG and RP.

Materials and Methods
RP and NG were introduced in an all-in-all-out nursery (d0) at approximately 20 and 40 days of age, respectively. They were transferred to a grow-finishing (GF) unit at d45 of the study. A total of 24 piglets were selected, 12 NG and 12 RP. Blood was collected at d0, d6, d13, d20, d27, d42, d48, d55 and d95. Selected piglets were housed in 6 pens (3 for NG and 3 for RP) of 4 piglets in three different rooms of a capacity of 72 piglets each. NG were in separate pens but they had nose-to-nose contact with RP through the fence. All 24 piglets were weighed and scored for clinical signs (1: normal; 2: unthrifty; 3: sick) at each sampling day of nursery. Presence in serum of PRRSv and antibodies against PRRSv were tested by a quantitative RT-PCR (qPCR) and IDEXX-2XR ELISA on all samples. Serum protein electrophoresis1 and complete hematologic profile were done on most sampling days. Finally some sera (d6, d13, d27 and d42) were screened for influenza H1N1 and H3N2 antibodies (IHA). Differences between groups of pigs, sampling days and interaction were tested with mixed models2.

Results
Viremia for PRRSv (qPCR positive) was observed in only 2 RP at d0 and in 3 RP at d20. From d42 to d48, most piglets became viremic (PRRSv peak), but no clinical signs were observed on either group of pigs during this period. All pigs had seroconverted (ELISA) by d95. Clinical signs (mainly dyspnea and unthriftiness) were observed between d13 and d20 on most piglets and were found to be related to the circulation of H3N2 influenza virus (paired sera). Clinical signs were less severe in NG. However, piglets in both groups had similar nursery growth rates (ADG=0.487 kg).

Most hematological parameters varied over time in both groups but the dynamics were different between groups. The main variations were observed for cellular immunity and globulins (see table 1).

Discussion
Almost no viral circulation of PRRSv was observed among selected piglets until the end of the nursery period (d42); infection of NG occurred later than expected even though there was nose-to-nose contact with infected piglets. In this study, NG were less affected by the influenza virus than RP and showed different immune responses (cellular and humoral). It could be that NG had a more mature immune system than RP because they were older when introduced in the nursery. Also NG had a higher health status than RP; their immune resources were possibly more readily available to fight pathogens.

References

Table 1. Blood parameters for NG and RP

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>NG (Mean±SD)</th>
<th>RP (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cells</td>
<td>EXP</td>
<td>18±2</td>
<td>17±2</td>
</tr>
<tr>
<td>Hb (g/L)</td>
<td>EXP</td>
<td>16±1</td>
<td>15±1</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>EXP</td>
<td>7±2</td>
<td>8±2</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>EXP</td>
<td>7±1</td>
<td>7±1</td>
</tr>
<tr>
<td>Globulins</td>
<td>EXP</td>
<td>7±1</td>
<td>7±1</td>
</tr>
</tbody>
</table>

Comparison between groups: * p<0.05 ** p<0.01


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Time-course of the immune response to Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) in gilts

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Introduction
The aim of this study was to evaluate the time-course of the immune response to PRRS virus in two PRRS positive farms. Gilts came from a PRRS-free herd and the purpose was to house them without causing PRRS outbreaks in the two farms under study. For this reason, two different strategies were adopted: vaccination in farm 1 and direct contact with viremic animals in farm 2. The cell-mediated immune response was evaluated by a PRRS-specific interferon-γ release assay; also, an ELISA assay was performed to detect PRRS-specific IgA Abs in saliva, the assessment of which could be useful in association with other routine diagnostic tests.

Materials and Methods
Animals. Two PRRS-positive farms, hereunder named 1 and 2 were chosen for the study. Gilts came from a PRRS-free herd, 10 animals from farm 1 and 15 animals from farm 2, respectively. Pigs from herd 1 were primo-vaccinated i.m. with MLV Porsilis® PRRS Intervet at 5 weeks of age, and a second vaccination with KV Progressis® Merial was carried out by the same route on 5-months old animals. In herd 2, animals were housed with viremic pigs until they were 5 months old. In farm 1, blood samples were collected twice: T0, 60 days after MLV vaccination and T1, 60 days after KV vaccination. In farm 2, blood samples were collected three times: T0 (3-month old animals), T1 (5-months old animals during contact with viremic pigs), T2 (9-months old animals). Moreover, these animals were checked for PRRSV-specific IgA Abs in saliva samples at T1 and T2. Laboratory analyses. Blood samples were collected in tubes with and without lithium-heparin, in order to obtain both serum (for anti-PRRSV IgG, RT-Real Time PCR, anti-SIV Ab) and plasma samples (for IFN-γ assay). The IgG Abs ELISA was performed as recommended by the producer (Herdcheck® IDEXX Porcine Reproductive and Respiratory Syndrome Antibody Test Kits); moreover, sera were used in RT-Real Time PCR to detect PRRSV RNA (3) and inhibition of haemagglutination (IH) for influenza virus type A/SW/H1N1, A/SW/H1N2 and A/SW/H3N2 (4). After incubation of whole blood with PRRSV and mock antigens, IFN-γ was measured by sandwich ELISA using anti-swine IFN-γ capture mAb P2F6 and biotinylated, anti-swine IFN-γ mAb MP701B (Thermo Scientific, Rockford, IL). Swine IFN-γ was revealed by HRP-conjugated streptavidin, and ortho-phenilenediamine as substrate of the color reaction. Samples were scored positive if the OD corresponding to PRRS-stimulated whole blood was higher than that of mock-stimulated and unstimulated blood by 20 mOD at least. PRRSV-specific IgA in saliva samples was investigated by an ELISA assay; samples were reacted with sucrose-purified type I PRRSV and mock antigen, respectively. Mucosal Abs were revealed by an anti-swine IgA, HRP-conjugated antisera and ortho-phenilenediamine. Samples were scored positive if the OD corresponding to PRRSV antigen was higher by 20 mOD at least (background level shown in SPF pigs).

Results
Animals remained healthy until the end of the study. In farm 1 all pigs, but one, were PRRSV-negative in RT-Real Time PCR at T0 and T1, while they were IgG-positive at both T0 (except 1 animal) and T1; a PRRSV-specific IFN-γ response was absent at T0. Instead, there were 4 specific and 2 non-specific responses at T1. These animals also showed a seroconversion to H2N1 SIV. In farm 2, RT-Real Time did not reveal any PRRSV-positive sera; IgG ELISA indicated instead a seroconversion at T1 (except 1 animal), and low Ab titres at T2, too. The IFN-γ response was absent at T0; then, 3 PRRSV-specific and 3 non-specific reactions were scored at T1 and T2, respectively. The IgA ELISA assay revealed 10 and 3 positive reactors at T1 and T2, respectively, with an inverse frequency ratio to IgG reactors in serum.

Discussion
In both farms, the cell-mediated immune response occurred at low intensity as opposed to the Ab response. This finding confirms the results by other authors (1, 2), i.e. the discrepancy between humoral and CMI response to PRRSV following both vaccination and direct contact with viremic animals. Also, these preliminary data indicate a different time-course between IgA and IgG Ab responses, as a possible strategy of PRRSV to escape immune pressure.

References
Serological screening in PRRSV infection

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Introduction
Porcine Reproductive and Respiratory Syndrome was first described in 1980 in North America, following (from 1990) in Europe. From here PRRS occurs worldwide in most major swine-raising countries.

Both domestic and wild boars were the known susceptible species at PRRSV in natural condition.

There are kits to detect specific antibodies against PRRSV in serum samples. The most used kits are: indirect immunofluorescent antibody (IFA), ELISA and serum neutralization.

The formation and decline antibodies kinetic in IFA and ELISA are similar. The ELISA advantages are: rapid execution, low results variation comparing with results interprets by a technician.

Materials and Methods
For antibodies detection was samplings sows from breeding farm and from pigs from fattening unit that belongs to the anterior mentioned farm. From sows are sampling in the first period of gestation and from fattening pigs at 2, 3 and 5 months of age.

The trials were performed using PRRS IDEXX ELISA (HerdCheck IDEXX Laboratories Switzerland AG.), and xChek (Laboratories IDEXX) software.

Results and discussions
In the breeding farm were observed more reproductive disorders, and there were serological tested against PRRS.

Figure 1

The obtained results are presents in table 1. As it could be observed, in breeding farm and in fattening farm both are present the PRRS infection.

Table 1

<table>
<thead>
<tr>
<th>Farm 1 - breeding</th>
<th>Farm 3 - fattening</th>
<th>Farm 3 - fattening</th>
<th>Farm 3 - fattening</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st sampling</td>
<td>2nd sampling</td>
<td>3rd sampling</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>15</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>Negative samples</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Positive samples</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>11</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>Infection %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>75</td>
<td>70</td>
<td>91</td>
</tr>
</tbody>
</table>

In breeding farm, 80% from samples were positive. In fattening farm, at first sampling 75% samples were positive, at second sampling, 70% samples were positive and at third sampling, just after slaughtered, 91% samples were positive. This proved the seroprevalence rising.

Graphic 1. Dynamic of positive samples in fattening pigs

Conclusions
The breeding farm was positive in a significant proportion (80%). Also, all three samplings from fattening farm revealed positive reactions, as it follows: 75% at 2 months old, 70% at 3 months old and 91% at 5 months old.

Early identification of PRRS infected herds is essential for epidemiological surveillance and disease control. The herds moving is necessary to be done only between herds with the same immune status, through this disease spreading is limited.

References
Risk factors for the porcine high fever disease in a region of Vietnam

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Introduction
Porcine high fever disease (PHFD) emerged in 2007 as a large and rapidly evolving outbreak affecting most of China, and subsequently, Vietnam (1). The disease was characterized by fever, depression, anorexia, and respiratory distress; with high morbidity. One genotype of the porcine reproductive respiratory syndrome virus (PRRSV) was considered to be the etiological agent, possibly with involvement of other pathogens, because of the disease’s unusual characteristics (1). However an interaction between PRRSV and other pathogens was not confirmed. It is important to understand the epidemiological characteristics of PHFD. This study’s objective was to evaluate the distribution of, and risk factors for, the occurrence of PHFD in a region of Vietnam which underwent a recent outbreak.

Materials and Methods
Study area: The area of interest was a district of a province of southern Vietnam that had reported the outbreak of PHFD during 2008. A study area, approximately 10x10 kms in dimension, which contained 5 communes and a total of 37 hamlets, was selected.

Data collection: A survey was conducted in the study area to collect information about the swine health problems during 2008. A group of trained interviewers went to almost all households having pigs with local veterinarians to fill questionnaires, which included 3 sections: general information, clinical signs of disease in pigs, and production factors hypothesized to be risk factors.

Definition of case at household level: Based on clinical signs from the literature (1,2), and opinions of experts and experienced veterinarians in the area, cases were defined as households that experience a pig that died or aborted accompanied by one of the suspected signs, including: high fever (41.5°C), blue ear, respiratory problems in sows, stillbirths, pre- and post-weaning death up to 20%, or with mortality over 10% accompanied by fever and respiratory symptoms in other pigs.

Data analyses: Questionnaire data were entered into EpiData 3.1 (EpiData Association, Odense, Denmark). Logistic regression with a random intercept at the hamlet level was used to assess risk factors for PHFD at the household level using Stata 10 (Stata Corporation, Texas, USA).

Results
Of 955 households with questionnaire data, 33.4% were classified as cases. Cases occurred over the entire year (2008) with peak frequency in June. In addition to clinical signs defined

<table>
<thead>
<tr>
<th>Parameter (Parameter)</th>
<th>Coefficient</th>
<th>95% CI (lower)</th>
<th>95% CI (upper)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-1.50</td>
<td>-1.85</td>
<td>-1.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Log sow</td>
<td>0.39</td>
<td>0.28</td>
<td>0.51</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Log finishing pig</td>
<td>0.05</td>
<td>0.01</td>
<td>0.09</td>
<td>0.047</td>
</tr>
<tr>
<td>WGP * Duck WGP</td>
<td>0.04</td>
<td>-0.34</td>
<td>0.43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duck nct</td>
<td>-0.26</td>
<td>-0.70</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Ducks ct</td>
<td>0.18</td>
<td>-0.49</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>WGP-duck nct</td>
<td>0.63</td>
<td>0.01</td>
<td>1.26</td>
<td></td>
</tr>
<tr>
<td>WGP-duck ct</td>
<td>1.04</td>
<td>0.04</td>
<td>2.03</td>
<td></td>
</tr>
<tr>
<td>Receiving pigs</td>
<td>0.53</td>
<td>0.15</td>
<td>0.91</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Discussion
Number of sows and finishing pigs were used in the model to control for confounders. The final model fitted suggested that the movement of pigs (i.e. receiving nursery pigs form another farm) might be a risk factor with OR=1.70 (1.16-2.48). This factor might have contributed to the observed pattern of disease transmission over the area. The interaction between duck and feeding WGP suggested that potential pathogens originating from a water source, and which further multiplies in ducks, could contribute to occurrence of PHFD.

References
Risk assessment of PRRS outbreak in endemic farms according to productivity and health management variables

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Introduction
Porcine reproductive and respiratory syndrome (PRRS) is a devastating disease in intensive pig production and is an endemic disease in most farms in the world, but triggering factors for outbreaks should be studied for each scenario.

The objective of this study was to assess the factors linked with PRRS outbreak presentation in field conditions.

Material and Methods
Forty-three Spanish farms, belonging to the same company and with a census between 100 and 5,250 sows and gilts (mean±SD: 643±1,012) were surveyed during a minimum period of 3 years (and a maximum of 10 years) (total of 397 farm-years) in a prospective observational study.

Twelve variables related to farm facilities, stocking management, health status and strategies were studied.

Four different strategies for PRRS control were used in these farms: S1: PRRS-Positive Gilts, Live Vaccine and No Infection with Piglets; S2: PRRS-Positive Gilts, Live Vaccine and Infection with Piglets; S3: PRRS-Positive Gilts, Killed Vaccine and Infection with Piglets; and S4: PRRS-Negative Gilts, Killed Vaccine and Infection with Piglets. Live vaccine was AMERVAC®-PRRS (HIPRA) and killed vaccine was PROGRESSIS® (Merial). They were applied according to producer specifications. A commercial indirect-ELISA test was used to confirm PRRS diagnosis (Ingezim PRRS K1, Ingenasa).

In this study, a PRRS outbreak was defined as the sudden occurrence of reproductive problems associated to an abrupt increase of seroprevalence to this disease. Comparison of outbreak frequencies was performed with Likelihood Ratio test (for more than 2 frequencies) or Fisher’s exact test (for 2 frequencies). Relative Risk and 95% confidence interval were calculated for significant associated variables. Error α was established at 0.05. All statistical analyses were carried out with SPSS® 15.0 for WINDOWS®.

Results and Discussion
During the study period only 7 PRRS acute outbreak were recorded. The only variable significantly associated with outbreak risk was the stock variation (see Table 1), so an increase of census led to a higher probability of PRRS outbreak occurrence (40%) versus a probability of 1.3% when no changes in census; that corresponded to a significant Relative Risk of 31.2 (95%CI: 7.8 – 124.3). It means, that there is 31.2 times more risk for PRRS outbreak to occur in farms that undergo a relevant increase of population size, than in farms with constant census.

Other variables had not been associated with PRRS outbreak (Table 1). In this study, we didn’t find any significant impact of the type of vaccine used (live or killed) on the outbreak risk.

However several variables could be related to outbreak risk, but the very low number of outbreaks did not allow us to get significant results (i.e. existence of infected piglets in post-weaning, use of outsource gilts and presence of in-farm AI station).

Table 1: Relationship between productivity & health management variables and PRRS acute outbreak

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>N</th>
<th>Outbreak</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fattening unit</td>
<td>Absence</td>
<td>328</td>
<td>1.8%</td>
<td>&gt;0.9991</td>
</tr>
<tr>
<td></td>
<td>Presence</td>
<td>69</td>
<td>1.4%</td>
<td></td>
</tr>
<tr>
<td>Post-weaning unit</td>
<td>Absence</td>
<td>10</td>
<td>0.0%</td>
<td>&gt;0.9991</td>
</tr>
<tr>
<td></td>
<td>Presence</td>
<td>387</td>
<td>1.8%</td>
<td></td>
</tr>
<tr>
<td>In-farm AI station</td>
<td>Absence</td>
<td>377</td>
<td>1.9%</td>
<td>&gt;0.9991</td>
</tr>
<tr>
<td></td>
<td>Presence</td>
<td>20</td>
<td>0.0%</td>
<td></td>
</tr>
<tr>
<td>In-farm gilts rearing</td>
<td>No</td>
<td>358</td>
<td>1.7%</td>
<td>0.518</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>39</td>
<td>2.6%</td>
<td></td>
</tr>
<tr>
<td>Stock variation</td>
<td>Decrease</td>
<td>2</td>
<td>0.0%</td>
<td>&lt;0.0011</td>
</tr>
<tr>
<td></td>
<td>No changes</td>
<td>390</td>
<td>1.3%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increase</td>
<td>5</td>
<td>40.0%</td>
<td></td>
</tr>
<tr>
<td>Gilt source</td>
<td>Out</td>
<td>363</td>
<td>1.9%</td>
<td>&gt;0.9991</td>
</tr>
<tr>
<td></td>
<td>In</td>
<td>34</td>
<td>0.0%</td>
<td></td>
</tr>
<tr>
<td>PRRS status of gilts</td>
<td>Negative</td>
<td>178</td>
<td>2.2%</td>
<td>0.7052</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>219</td>
<td>1.4%</td>
<td></td>
</tr>
<tr>
<td>Gilt infection with piglets</td>
<td>No</td>
<td>69</td>
<td>1.4%</td>
<td>&gt;0.9991</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>328</td>
<td>1.8%</td>
<td></td>
</tr>
<tr>
<td>Type of vaccine for gilts</td>
<td>Live</td>
<td>164</td>
<td>1.2%</td>
<td>0.7052</td>
</tr>
<tr>
<td></td>
<td>Killed</td>
<td>233</td>
<td>2.1%</td>
<td></td>
</tr>
<tr>
<td>Killed vaccine use</td>
<td>Only gilts</td>
<td>357</td>
<td>1.4%</td>
<td>0.150</td>
</tr>
<tr>
<td></td>
<td>All breeders</td>
<td>40</td>
<td>5.0%</td>
<td></td>
</tr>
<tr>
<td>Infected piglets in post-weaning</td>
<td>No</td>
<td>26</td>
<td>0.0%</td>
<td>0.289</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>46</td>
<td>8.7%</td>
<td></td>
</tr>
<tr>
<td>Health strategy for gilts</td>
<td>S1</td>
<td>67</td>
<td>1.5%</td>
<td>0.896</td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>96</td>
<td>1.0%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S3</td>
<td>54</td>
<td>1.9%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S4</td>
<td>177</td>
<td>2.3%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>397</td>
<td>1.8%</td>
<td></td>
</tr>
</tbody>
</table>

1 Likelihood Ratio test p-value; 2 Fisher’s exact test p-value

Conclusion
Increase of the population size is a critical risk factor for acute outbreak in farms with endemic PRRS independently of implemented health strategies.

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Correlation between genetic, temporal and geographic distances of porcine reproductive and respiratory syndrome virus (PRRSv) strains isolated in swine herds from a high density area in Quebec, Canada

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Introduction

The transmission of PRRSv between herds can occur over long distance by the introduction of infected animal or semen (1), but also over short distances by mechanisms such as aerosols or various mechanical/biological vectors (2). Short distance processes of transmission could be largely enhanced in high density production area, complicating PRRS control at a herd level. PRRSv strains sharing a high level of homology are suggestive of a common source of infection, and thus can be used to explore the temporal and geographical spread of the virus. The objective of our study was to determine the correlation between genetic, temporal and geographical distances of PRRSv strains in a high density area of swine production.

Materials and Method

As a part of a larger study on the transmission and control of PRRS, a census of production sites was conducted in 10 municipalities located in a high density area (Monteregie) between February 2005 and June 2007. PRRS herd status was determined by ELISA and qRT-PCR and when PCR positive, sequencing of ORF5 was performed. For each production site, geographical coordinates were obtained using GPS at the closest building from public road. A pairwise alignment of all ORF5 sequences obtained was generated in Bionumerics software and genetic distances were calculated from nucleotides. Euclidian and temporal distances were calculated in SAS (SAS Institute 1989). Mantel test procedure was computed in R software to examine the correlation among genetic, geographical and temporal distances. P-values were computed based on 999 permutations.

Results

PRRS herd status was determined on 191 of the 200 participating sites, of which 176 were positive for PRRSv. One sequence was identified for 132 production sites, which were attended by 25 different veterinarians. Independent and integrated producers (7 production systems) accounted for 61% and 39% of the total. Sequences were identified on farrowing/farrow-to-finish and on weaner/finisher sites in a proportion of 58% and 42%, respectively. Table 1 shows descriptive results of pairwise distances included in the distance matrices used to compute the Mantel test. The bivariate Mantel test revealed a significant association between genetic and temporal distances ($r_M=0.06$ $p=0.04$). No significant relationship was found between geographical distance and either genetic ($p=0.68$) or temporal ($p=0.60$) distances.

Discussion

The correlation observed between genetic and temporal distances revealed that sequences isolated far away in time showed more genetic diversity, suggesting possible genetic evolution of the virus during the study period. The absence of correlation between genetic and geographic distances as previously reported (3) suggests that many processes could be involved in the between-herd transmission of the virus at both short and long distances. Further analyses will be performed to take into account other factors such as corporate organization membership.

References


Table 1: Descriptive results of pairwise distances between production sites included in the continuous distance matrices (n=8646)

<table>
<thead>
<tr>
<th>Distance variable</th>
<th>mean</th>
<th>sd</th>
<th>min - max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euclidean (m)</td>
<td>14957</td>
<td>8458</td>
<td>43-45726</td>
</tr>
<tr>
<td>Sampling time (day)</td>
<td>214</td>
<td>159</td>
<td>0-852</td>
</tr>
<tr>
<td>Genetic (%)</td>
<td>12</td>
<td>3</td>
<td>0-19</td>
</tr>
</tbody>
</table>
Risk factors for introduction and spread of PRRSV and PCV2 in pig farms in Korea

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National Veterinary Research & Quarantine Service, Anyang, Korea

Introduction
Although Porcine Reproductive and Respiratory Syndrome (PRRS) and Porcine Circovirus Disease (PCVAD) induce severe economical losses in domestic pig farms, most research are concentrated on the diagnosis of the causative agent and there is a need for more studies on epidemiological analysis of risk factors related to the transmission of these diseases. In this study, we conducted a survey to identify risks factors for PRRS and PCV2 infection in pig farms in Korea.

Materials and Methods
Survey was conducted from July 2008 to December 2009 through on-farm visits and use of questionnaires which were designed based on risk factors identified during previous epidemiological investigations conducted for Foot-and-Mouth Disease and Classical Swine Fever. Also, swab samples were collected from various key areas and equipments in the participating farms and tested to identify the degree of PRRS and PCV2 contamination during each season in 2009. Samples were tested using RT-PCR and nested PCR.

Results
A total of 58 pig farms in 9 provinces were surveyed. In our survey, the percentage of farms that disinfected the farm entrance, pig house entrance, inside of the pig house and farm surroundings were 36%, 26%, 22% and 16%, respectively. For tools, equipment and feces removal vehicles, disinfection was conducted in 93%, 93% and 69% of the farms, respectively when entering the farms. In contrast, disinfection was conducted in only 42%, 38% and 42% of the farms, respectively when leaving the farms. For refrigerators used to store veterinary drugs, 29% of the farms responded that they conducted disinfection.

Samples were collected from pig farms during spring, summer, autumn and winter, which were totals of 6, 14, 24 and 21 farms, respectively. The percentage of farms where PRRSV and PCV2 were detected were 83.33% and 10.00%, respectively during spring, 3.35% and 16.32%, respectively during summer, 19.62% and 38.06%, respectively during autumn, and 39.94% and 54.45%, respectively during winter. In particular, investigation of refrigerators in 6 pig farms during each season showed PRRSV and PCV2 contamination in 100% and 33.3% of the farms, respectively during spring, 0% and 16.7%, respectively during summer, 0% and 66.7%, respectively during autumn, and 0% and 80.0%, respectively during winter. Viruses were consistently detected from shoes, vehicles or various tools and equipment such as wheel barrows and shovels used in the farms, although there were slight variations depending on the season. Also, there was persistent detection of viruses from the floor of pig houses.

Discussion
This study demonstrated that pathogens can be transmitted during the movement of people or equipment associated or used in pig farms, and the rates of detection of these two viruses were different according to the season. Refrigerators could become an important source of diseases as viruses in refrigerators would be less influenced by the difference in the season. On a positive note, the rates of detection of viruses were lower on the secondary tests after the farm had been educated, than on the primary investigation. The method used in this study including sample collection and epidemiological investigation can be applied to the standard surveillance for porcine diseases conducted in Korea. Widespread use of this method in the porcine industry would result in a more scientifically sound surveillance allowing for more effective disease control measures to be implemented.

References
**Spatiotemporal distribution of the dominant PRRSV genotype in Ontario**

**Thomas Rosendal; Cate E. Dewey; Robert M. Friendship; Sarah K. Wootton; Young Beth; Zvonimir Poljak**

*University of Guelph, Guelph, ON, Canada*

**Introduction**

Modeling the spatiotemporal distribution of porcine reproductive and respiratory syndrome virus (PRRSV) is important in the pursuit to understand how PRRS spreads in a population of pig herds. The distribution in space and time of PRRSV describes part of a set of connections between herds. The objectives of this study are to search for trends and clusters in space and time of a common genotype of PRRSV in Ontario and to assess the importance of ownership structure in of PRRSV genotypes in Ontario.

**Methods**

Herds were eligible for inclusion in the study if a PRRSV positive diagnostic sample was identified at the Animal health Laboratory at the University of Guelph between Sept 2004 and Aug 2007. Herd managers who were willing to participate were interviewed to determine herd ownership. The ORF5 gene of the PRRSV positive sample was sequenced and the RFLP type according the University of Minnesota method was determined. Spatial and temporal autocorrelation and clustering of the most common type, Minnesota RFLP type 1-18-4, were investigated using the space-time k-function and the SaTScan Bernoulli model. The distribution in space and time was modeled using the generalized additive model (GAM). The effect of ownership on the space-time distribution was tested using fixed effects in the GAM for the owners with the largest number of members.

**Results**

The k-function showed that autocorrelation was present in PRRSV type 1-18-4 up to a distance of 6 km and 45 days as well as a distance of 12 km and 15 days. A cluster of cases was found in the eastern part of the study period lasting 4 months from Nov/2005 - Feb/2006 (P=0.046). A trend in the spatial distribution of type 1-18-4 was found using the GAM (P=0.001) and no significant temporal trend was found (P=0.09). An association was found between ownership of herds and the presence of type 1_18_4 (P=0.005). When ownership was added to the space-time model the shape of the spatial trend did not change. Two owners had OR= 7.3 (P=0.004) and OR=6.7 (P=0.02) of having type 1-18-4, where small owners, those with less than 6 herds in the study, were at reduced odds, compared to large ownerships, of having type 1_18_4 (OR=0.12, P<0.001).

**Discussion**

The autocorrelation in cases of 1-18-4 indicates that herds up to 6 km from a case herd are more likely to be cases if tested within 45 days of each other but also out to as far as 12km if they are tested within 15 days of each other. Type 1-18-4 was also not uniformly distributed in Ontario during the study period (Fig 1a). Although, some of the variation can be explained by the ownership structure, a spatial trend remains after accounting for ownership (Fig 1b). The shape of the spatial trend did not change after accounting for ownership; this is an indication that the spatial effect and the effect of ownership are not confounded, meaning that the spatial effect is not explained by ownership. Further investigation is needed to shed light on the origin of this trend. The spatial pattern could be a result of similarities of breeding stock, animal or semen suppliers between herds or be an indication of transmission between herds. The variables describing herd suppliers could not be adequately accounted for using these methods and future work will address these connections.
Association between genetic similarity of the PRRSV ORF5 sequence and the similarity in clinical signs of PRRS in Ontario swine herds

Thomas Rosendal; Cate E. Dewey; Robert M. Friendship; Sarah K. Wootton; Young Beth; Zvonimir Poljak

University of Guelph, Guelph, ON, Canada

Introduction
Porcine reproductive and respiratory syndrome (PRRS) is a clinically variable disease. Clinical signs that are recognized by the producer include abortion and anorexia in sows, stillborn and mummified piglets, dyspnea, respiratory disease and increased mortality in all ages of pigs (1). Isolates of PRRSV are genetically variable in the ORF5 gene. The association between clinical disease and the ORF5 gene have been infrequently investigated under field conditions (2). The objectives of the present study were to measure the association between RFLP type and clinical signs of PRRS in the herd and to assess the association between clinical similarity between herds and the similarity of PRRSV isolates from the herds.

Materials and Methods
Herds were eligible for the study if a PRRSV PCR test result from the Animal Health Laboratory at the University of Guelph was found between Sept 2004 and Aug 2007. Herd managers were surveyed about clinical signs of PRRS in the herd and ORF5 of the PRRSV isolate was sequenced. The RFLP type of the virus was determined and the genetic similarly of all virus pairs was calculated. The similarity of each clinical sign was determined to be either similar or dissimilar for all pairs of herds.

The association between RFLP type and clinical disease was tested by regressing each clinical sign on the RFLP types using logistic regression (P<0.05 was significant). The association between sequence similarity and clinical similarity was tested by the Mantel test and the generalized additive model where the outcome was similarity of a given clinical sign and the independent variable was the similarity of the ORF5 gene.

Results
RFLP type 1-0-4 was associated with increased probability of abortion, sows off-feed, stillbirths, weak-born pigs, sow/boar mortality, and preweaning mortality. RFLP type 1-0-2, was associated with clinical disease in the farrowing phase of production and type 184 was significantly associated with increased abortions.

The GAM models indicated a significant association between PRRSV sequence similarity and similarity of both abortions and stillbirths and that the chance of similarity of these clinical signs was significantly increased when sequences were >92% similar (Fig 1). Including vaccine like viruses in the analyses caused similar model to decline at the upper end of homology.

Discussion
These results indicate that variation in clinical signs of PRRS is caused in part by variation in the PRRSV. For abortions and stillbirths, PRRSV isolates of >92% homology of the ORF5 gene are associated with similar clinical signs. This is in contrast to the benchmark where viruses of <98% ORF5 sequence similarity are considered different (3). Presence of vaccine type virus in a population can have an impact on the interpretation of PRRSV epidemiology. Removal of vaccine type observations before analysis may be necessary to interpret studies such as this because vaccine isolates are genetically similar but are not expected to be associated with similar clinical signs of disease.

References
Clinical and virological characterization of European-type PRRSV isolates of high and low virulence

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Introduction
Porcine Reproductive and Respiratory Syndrome (PRRS) is a swine disease produced by PRRS virus (PRRSV), which is antigenically and genetically heterogeneous (1). PRRSV variability extends also to pathogenicity and highly and moderately virulent isolates have been described within the American genotype (2). The infection by highly virulent isolates produces severe clinical signs, significant gross lung lesions and high levels of viremia (2, 3). In a previous study, we characterized the pathogenicity of seven European-type isolates including one isolate which virulence was comparable to that described for American type isolates (4). The aim of this study was to compare the pathogenic properties of this isolate with that exhibited by a European type isolate of remarkable low virulence.

Materials and Methods
Two European-type isolates were used in this study. Additionally a modified live vaccine strain, Ingelvac PRRS, was used as an attenuated control. European isolates were propagated in porcine alveolar macrophages (PAM) and the fifth passage was used for pig inoculation. Vaccine strain was used directly from the bottle. For pig inoculation, a total of 45 3-week old piglets seronegative to PRRSV were divided into 3 groups. On Day 0 of the experiment piglets of groups A, B and C were inoculated intranasally (IN) with 5 x 10^5 TCID50 of highly virulent isolate, low virulent isolate and vaccine strain, respectively. All pigs were examined daily for clinical signs and rectal temperatures were recorded until the end of the experiment. Biological samples, including serum, nasal and rectal swabs were collected every three days. Five piglets per group were sacrificed on days 7, 14 and 21 post-infection (p.i.) and different tissue samples were collected. At necropsy macroscopic lung lesions were evaluated. Determination of viremia and organic distribution were carried out by virus isolation as previously described (5) on cultures of PAM in the case of field isolates and MARC 145 in the case of vaccine strain. Clinical and viremic results for each pig were converted to an approximate area under the curve (AUC) and a complete statistical study was carried out to estimate differences between groups. Finally, a cluster analysis of clinical and virological parameters was performed.

Results
After inoculation, severe clinical signs were observed in pigs exposed to highly virulent isolate, leading to a AUC values of 5.13, 8.0 and 0.4 on the first, second and third week p.i. On the contrary, pigs infected with low virulent isolate and vaccine strain remained clinically normal throughout the experiment, reflected in AUC values that never exceeded 0.5. Seemingly, fever was only recorded in pigs exposed to highly virulent isolate, namely from day 2 p.i. to day 10 p.i. Consistently with the clinical score macroscopic lung lesions were more severe in pigs inoculated with the highly virulent isolate.

Looking at the virological parameters, viremia was more intense with statistically significant differences in those pigs inoculate with highly virulent isolate. Additionally, highly virulent virus was isolated more frequently from tissue samples than low virulent or vaccine strain, being viral load also higher in the former group. Finally, remarkably low virulent isolate was never detected in any swab, indicating a very low shedding rate.

The apathogenic phenotype of the low virulent isolate was confirmed by the grouping of pigs of this group with those infected with the vaccine strain in the cluster analysis.

Discussion
This is the first description of the existence of highly pathogenic and apathogenic PRRSV isolates within the European type. The ultimate causes of the differences in pathogenicity between these two isolates were not elucidated in our study. However, there are strong indications that the replication rate of the isolates and the organic distribution in the pigs upon inoculation can determine their virulence.

Acknowledgements
This study was supported by grant CSD-2006-00007 (Consolider Ingenio 2010).

References