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Investigation of risk factors for porcine circo virus type 2 (PCV2) following an outbreak of Post-weaning Multi-systemic Wasting Syndrome (PMWS) on a large commercial pig farm

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Introduction

Porcine circo virus type 2 (PCV2) has been widely acknowledged as the necessary agent for post-weaning multi-systemic wasting syndrome (PWMS) in pigs. Various studies have identified potential risk factors, but it is still not fully understood what triggers the onset of this multi-factorial disease.

The present study aimed at evaluating the presence of porcine circovirus 2 (PCV2) and other pathogens before and during an outbreak of PMWS on a large commercial pig farm in the UK. Further we investigated the effect of environmental conditions and the parity and PCV2 immune and infection status of sows on presence of PCV2 in growing pigs.

Material and Methods

The study was based on data collected in two different research projects ongoing on a large commercial farm when a PWMS outbreak hit the farm. One project was a large scale experimental study on the impact of environmental conditions (dust and ammonia) on the onset of respiratory disease in 960 weaned pigs divided in 8 batches, batches were run one at a time over 2 years. At the end of the 42 day exposure experiment, serum samples were collected and post-mortem carried out on randomly selected pigs. The other project was a longitudinal study on the reproductive performance during the first three parities and involved 188 gilts originating from eight different breeders. Serum samples of 371 growing pigs, aged 10-12 weeks, were tested for antibodies to PCV2 and other endemic pig pathogens. In addition, presence of PCV2 antigen was determined by semi-quantitative PCR. Fifty gilts and sows produced 135 of the 371 growing pigs of which serum samples had been collected. These 50 gilts and sows had been repeatedly sampled at different time points in the reproduction cycle resulting in a total of 296 samples which were tested for PCV2 antibodies and PCV2 antigen.

Results

Results show that PCV2 sero- and PCR-positive pigs were already present on the farm long before the actual PMWS outbreak. Prevalence of PCV2 antibodies and antigen in growing pigs was low in the first batches of the respiratory experiments, but reached up to 100% in the late batches. The weight gain in PCV2 PCR positive pigs was significantly lower than in negative pigs (p<0.01) and PCV2 PCR positive pigs had a significantly higher overall pathological score in the post-mortem examination with a mean of 10.8 compared to 8.5 in PCR negative pigs (p<0.01).

In sows, differences in sero-prevalence was observed for different stages in the reproduction cycle and sows in the second half of gestation and around farrowing seemed more likely to have measurable titres of PCV2 antibodies. Over a period of two years, most sows showed fluctuations in PCV2 antibody titres. When combining laboratory results of sows and growing pigs, growing pigs had higher odds to be PCV2 PCR positive if the sow tested positive for PCV2 antibodies in the time period around farrowing (OR 24.5, p=0.01), the sow was of parity 3 or higher (OR 40.9, p<0.01) and were less likely to test PCR positive if the sow originated from certain breeders (OR 0.04, p<0.01).

Discussion

Through previous research it is well understood that maternal PCV2 antibodies prevent onset, or delay onset, of PMWS in the weaner. The results of this study further highlight the role of the sow for PCV2 in weaners (parity, PCV2 status around farrowing and breeding background). Our results indicate that in future studies on PMWS more sow data should be included. The trend of increased sero-prevalence in the second half of gestation and around farrowing may indicate latent PCV2 infection in sows and that increased stress levels result in reactivation of PCV2, followed by the observed PCV2 antibody response.
PCV2 in extended pooled semen from US boar studs

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Introduction

Prior studies have demonstrated PCV2 DNA and the virus itself in raw and extended semen from healthy naturally or experimentally infected boars [1-4]. To provide an epidemiological context to this finding, our group recently reported that of 472 German and Austrian raw semen samples screened for PCV2, 86 (18.2%) were found to be positive [4]. In our continued work to provide a more global perspective of the epidemiology of PCV2 in boar semen, a cross-sectional study was performed to determine the occurrence of PCV2 in extended semen produced in US boar studs.

Materials and Methods

Randomly selected extended pooled semen samples (n = 198), 18-30 hours post-processing, originating from 17 studs located in different states of the US were tested for PCV2 using a nested PCR technique [4]. Extended-chilled samples submitted to the Andrology Laboratory at the University of Pennsylvania were centrifuged at 4,000 rpm for 10 min to produce sperm-rich and supernatant fractions. Fractions were cataloged and then stored at -20 degree C until subsequent analysis. For all sample positives, all fragments obtained with the internal nested PCR were additionally sequenced to confirm PCV2 identity. A chi-square test was used to determine differences (P < 0.05).

Results

A total 49 (24.7%) extended pooled semen samples were PCV2 positive (Table 1). Samples from 5 studs were negative; all other studs had at least one positive sample (range: 10 – 50%). Positive results were more often obtained with the sperm-rich pellet (n = 34) than with the supernatant (n = 8). A small number of samples (n = 7) were positive for both the sperm-rich pellet and corresponding supernatant.

Discussion

This study confirms that PCV2 is shed in semen and can still be detected after semen is extended and stored for 18-30 hours. The percentage of samples found positive corresponds well with previous results from North America and Europe [1–4]. Of particular interest in the present data set was the differences observed between studs. Also, the fact that most samples were positive in sperm-rich pellets may indicate that the virus is sperm associated rather than a loose component in seminal plasma. All semen samples tested in this study were pooled semen originating from several boars. It is thus not known if one or more boars contributed to the PCV2 contamination. Also virus quantification was not performed which might have been necessary in order to better determine the risk of venereal transmission.

Table 1. Results of analysis of extended semen samples for PCV2 by nested PCR and sequencing (n = 198)

<table>
<thead>
<tr>
<th>Stud ID</th>
<th>Pellet</th>
<th>S-tant*</th>
<th>Both#</th>
<th>Total</th>
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<tr>
<td>(n)</td>
<td>n</td>
<td>n</td>
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<td>0</td>
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<td>0</td>
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<td>0</td>
<td>1</td>
<td>0</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>4 (21)</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>4 (19.0)</td>
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<td>5 (34)</td>
<td>4</td>
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<td>4</td>
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</tr>
<tr>
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<td>5</td>
<td>4</td>
<td>0</td>
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<td>1</td>
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<td>Total</td>
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<td>8#</td>
<td>7#</td>
<td>49 (24.7)</td>
</tr>
</tbody>
</table>

*Supernatant; #Both supernatant and pellet were PCV2 positive; (a,b) P < 0.05.

References

Use of IgG and IgM differentiation of antibodies to detect primary and secondary immune response to Porcine circovirus type 2 (PCV2)

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Introduction

PCV2 is the causative agent for porcine circovirus associated diseases (PCVAD), which clinically affect pig herds worldwide.¹ As antibody titres are usually high in affected and in not affected herds serological tests are not suitable for laboratory diagnostics for PCVAD. Recently commercial vaccines to protect pigs from PCVAD became available.³ In this study IgG and IgM type antibodies to PCV2 in vaccinated and not vaccinated pigs were examined to demonstrate the effective priming of the immune system for contact to the pathogen.

Materials and Methods

In a GCP conform field trial on a Japanese pig farm a group of animals were vaccinated against PCV2 at 3 weeks of age (Ingelvac CircoFLEX®, Boehringer Ingelheim). A not vaccinated control group was housed commingled. Blood samples from 20 randomly selected pigs each from both groups were taken at the time of vaccination (3 weeks of age) and 4, 6, 8, 10 and 12 weeks after vaccination. All samples were tested for antibodies specific to PCV2 ORF2 antigen using a commercially available ELISA kit (INGEZIM CIRCOVIRUS IgG/IgM ELISA, Ingenasa, Madrid, Spain). The ELISA was performed according to the manufacturers instructions. In addition PCV2 genome load was quantified in the samples using a quantitative real-time PCR according to the method published by Brunborg et al.⁴

Results

Results of the tests are summarized in figure No. 1. Four weeks after the vaccination in both groups two animals were positive in the IgM specific ELISA. At this time the first samples from the control group were detected to be positive for PCV2 virus genome in the qPCR. In the following weeks the percentage of viremic animals in the control group increased. In parallel the number of IgM positive pigs rose in the control group. In the vaccinated group no more IgM positive samples were detected, but the number of IgG positive samples rises. Twelve weeks after vaccination still three samples from the control group were positive for IgM. The number of IgG positive samples was comparable in both groups at this time.

Discussion

The results presented in this study clearly demonstrate the effective priming of the porcine immune system after vaccination against PCV2. Not vaccinated pigs react against field virus exposure with the production of IgM (primary immune response), whereas vaccinated pigs react with a secondary immune response (predominantly IgG). The detection of many IgM positive animals in a group of pigs indicates a primary immune response. As the majority of vaccinated pigs does not react with IgM, but with IgG (secondary immune response) to clinical exposure to PCV2, the differentiation of IgG and IgM against PCV2 could be used as a diagnostic tool to differentiate not vaccinated groups of pigs from vaccinated ones.

References

**Diagnosis of Porcine Circovirus Diseases (PCVDs) by serology and qPCR**

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**Introduction**

Porcine circovirus type 2 (PCV2) is considered the essential infectious agent of postweaning multisystemic wasting syndrome (PMWS). PCV2 is an ubiquitous virus and most if not all pigs become infected during their life, but only a proportion of them develop PMWS (1,2). A threshold of 7 log10 PCV2 DNA copies/ml serum has been suggested to establish a PMWS diagnosis (2-4), however limited information are available on the use of PCR in the diagnosis of other PCV2 related diseases (PCVDs). The present study describes the results of serological and virological profiling from 14 Danish herds with symptoms of PCVD.

**Material and Methods**

Sixteen Danish swine herds were included. Fourteen of herds had one or more of the following clinical symptoms in weaners, growers and/or finishers: Pneumonia, diarrhea or unspecific symptoms and/or wasting. None of the herds vaccinated the pigs against PCV2. The last two herds were control herds without any symptoms of PCV2 related diseases. In each herd, information on production type, health status, number of pig produced, mortality, weight gain, antibiotic usage and clinical symptoms were recorded. Blood samples were taken from 5 pigs from each of 6 age groups: 3-4 weeks after weaning, 2 weeks before transfer to the fattening unit, 1-2 weeks and 7-8 weeks after transfer and finally just before slaughter. Serum was separated and kept at -80°C until test for antibodies against PCV2 using an in house ELISA and for PCV2 virus load by qPCR (5).

**Results**

Several different serological and virological profiles were found in the 16 herds, however basically the profiles could be grouped into two groups represented by herds 5 and 11 (figure). This grouping was independent of production type, size, health status etc. Apart from one herd, no virus was found in the samples taken 3-4 weeks after weaning. In the group of herds represented by herd 5, PCV2 viraemia and increased level of PCV2 antibodies were observed in weaners. In the other group of herds, represented by herd 11, viraemia and increased levels of antibodies were seen in older pigs – either in growers early after transfer or in finishers. In general, increased levels of antibodies were concurrent with increased PCV2 load. The level of antibody and virus in serum were very uniform within the sampling groups. The level of virus in serum were in most of the viraemic pigs between 4-6 log10 DNA copies/ml, however, a few pigs in some groups had higher levels (7-9 log10/ml). In some herds increased viral load in serum were seen in the groups of pigs concurrent with appearance of clinical signs whereas no correlation was seen in other herds. Furthermore, comparable levels of viraemia were also seen in the two control herds.

**Discussion and conclusion**

The present study showed that PCV2 profiles on serum for the diagnosis of PCV2 related diseases in herds can be a valuable tool to confirm that PCV2 is circulating. However, the results of the profiling should be carefully accessed and related to herd management factors, the presence of other pathogens and the clinical symptoms. Increased serum PCV2 load in itself are not diagnostic for clinical PCVD.

**References**

Comparison of PCV2 viremia between heavy and light weight pigs at marketing age in farms with routine PCV2 vaccination

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Introduction

Because of the economic significance, PCV2 vaccine is now routinely used on most US swine farms. Although vaccinated pigs perform significantly better in terms of survivability and growth performance, subpopulations of light weight pigs in late finishing phase still are observed in farms with routine PCV2 vaccination. Swine producers and veterinarians have expressed concerns over this problem and questioned whether protection by the PCV2 vaccine may be inadequate to prevent poor growth in some cases. Therefore, it is hypothesized that a protection of light weight pigs could be attributed to PCV2 infection at late finishing age. This preliminary study was designed to compare PCV2 viremia and the antibody status of the lightest weight and the heaviest weight pigs at the marketing age in farms that routinely vaccinated against PCV2.

Materials and Methods

Seven different finishing farms that routinely used a commercial PCV2 vaccine and were experiencing no evident PCV2 associated clinical disease were selected. Within 2 weeks before the marketing on each farm, 30 lightest and 30 heaviest pigs in a group were identified visually by each farm manager, and blood samples from these 60 pigs in each farm were collected. PCV2 IFA titer was tested by a protocol used routinely in our laboratory. A differential nested PCR assay for PCV2a and 2b was conducted as the method previously described¹. For PCV2b specific real-time PCR, a PCV2b specific primer set was used, and the assay was performed using PerfeCTa SYBR Green SuperMix and Mx3005P. One-way repeated measure ANOVA and Student t-test was used to test for statistical significant differences.

Results

Overall, the IFA titers of the light pigs were higher than those of the heavy weight pigs (Fig. 1). Percentages of PCV2a and PCV2b viremic pigs in the 7 different finishing units tested by nPCR are shown in Table 1. There was no significant difference in PCV2a viremic pigs between the light and the heavy weight groups. However, percentages of PCV2b viremic pigs were higher in the light pigs than the heavy pigs in 6 of the 7 farms. The real-time PCR results for the pigs in each farm showed that average amount of PCV2b genomic DNA was higher in the light pigs than in the heavy pigs.

Discussion

The present results indicate a potential association between PCV2 infection and the marketing weight in swine farms. Negative effects on production may still occur from PCV2 infection of vaccinated pigs when clinical diseases are not apparent. Although statistical significance was not always observed, higher PCV2 IFA titers and more viremic pigs were evident in the light weight pigs at marketing age. This finding also support a greater role of PCV2b compared with PCV2a suggesting that PCV2 vaccination should be focussed on the protection from PCV2b genotype infection. We could not determine when pigs may have been exposed to PCV2 on these farms but vaccination program may need to be adjusted to protect pigs from PCV2 infection until late in the finishing phase.

References

Distribution of ORF2 and ORF3 genotypes of Porcine Circovirus Type 2 (PCV2) in wild boars and domestic pigs in Germany

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Introduction
Porcine circovirus 2 (PCV2), the essential infectious agent in PCVAD (porcine circovirus associated diseases) circulates at high rates among domestic pig and wild boar populations. Wild boars may be viremic and shed the virus with excretions and secretions, and thus serve as a reservoir for domestic pig PCV2 infection. We hypothesize that PCV2 strains circulating in wild boars and in domestic pigs are significantly different and thus, partially independent. To prove this hypothesis, the distribution of ORF2 and ORF3 genotypes of PCV2 within wild boars and domestic pigs from overlapping greater areas of Germany was investigated.

Materials and Methods
Samples from 40 wild boars from 17 different hunting grounds and tissue samples of 60 apparently healthy domestic pigs from 18 provenances located in the same greater areas as the wild boars were analyzed. PCV2-specific DNA was amplified by nested PCR (nPCR). PCV2 genotypes were classified based on ORF2 sequences as described by Olvera et al. (2007) and Segalés et al. (2008). ORF2 and ORF3 genotypes were detected by pyrosequencing on a PyroMark ID (Biotage, Sweden) system. Genotypes were compared with PCV2 sequences from the Genbank database (520 entries).

Results
Two ORF2 and nine ORF3 genotypes were detected in twelve combinations, with significant differences between wild boars and domestic pigs. Distribution among the federal states did not differ significantly within species. PCV2-2b was the most common genotype in both species. Almost 60% of the infected wild boars but only 4.8% of the infected domestic pigs carried the PCV2-2a subtype. ORF3 genotype frequencies differed also significantly between wild boars and domestic pigs. Some ORF3 genotypes were detected in domestic pigs only, others exclusively in wild boars. The genotypes of domestic pig PCV2 samples were dominated throughout the country by 2b/ORF3-1 and 2b/ORF3-3 types. PCV2 genotypes of the wild boars were, however, more heterogeneous than those of domestic pigs. Two different PCV2 genotypes in one animal have been isolated from 27% and 4% of wild boars and domestic pigs, respectively (P<0.001), and at least two different PCV2 genotypes have been isolated from 90% of hunting grounds and 50% of domestic pig provenances. The five Genbank database PCV2 sequences from German domestic pigs (from 1999/2000) differed significantly from the “domestic” sequences of the present study (2004/2007) and from 124 sequences obtained from different European countries. However, European and the “domestic” sequences of the present study were in good agreement. The six Genbank sequences of European wild boar isolates agree with the major genotypes of the wild boars of the present study. Some of the wild boar derived genotypes are not present or very rare among “domestic” and wild boar Genbank sequences worldwide.

Discussion
Differences in “domestic” PCV2 genotypes of the present study and Genbank entries from Germany may indicate the general switch from PCV2-2a to genotype PCV2-2b after the year 2003 that has been generally described by Dupont et al. (2008). Differences and conformity of PCV2 genotypes derived from wild boars and domestic pigs, either from this study or from European Genbank database entries, indicate that wild boar and domestic pig PCV2 do coexist with some exchange. More than 50% of the wild boar PCV2 genotypes are extremely rare in domestic pigs in Europe (Genbank) and Germany (this study), even worldwide, inferring a certain independence of wild boar and “domestic” PCV2 genotypes. The fact that genotype 2a is prevalent in wild boars (57.5%), but rare in domestic pigs (4.8%) and that genotype 2b is the almost exclusive genotype in domestic pigs and also prevalent in wild boars, argues for the hypothesis that exchange of PCV2 between both species emerges primarily from domestic pigs to wild boars and less in the reverse direction. This hypothesis is supported by the finding that PCV2 loads of wild boars are significantly lower than those of domestic pigs (see abstract on the opposing page).

References