Proceedings of the 21st International Pig Veterinary Society Congress
IPVS
Jul. 18 – 21, 2010
Vancouver, Canada

Next congress:

22nd International Pig Veterinary Society Congress
June 10-13, 2012 – Jeju, Korea

Reprinted in IVIS with the permission of the IPVS
Effects of sugar cane extract and management improvement on PRRSV and PCV2 co-infection

WC Lee¹ JC Hsu¹ JW Liao¹ DY Lo² MS Chien¹ SL Hsuan¹ CJ Huang³ YL Huang⁴

¹Graduate Institute of Veterinary Pathobiology, National Chung Hsing University, Taichung, Taiwan; ²Department of Veterinary Medicine, National Chiayi University, Chiyi, Taiwan; ³Graduate Institute of Veterinary Microbiology, National Chung-Hsing University, Taichung, Taiwan; ⁴Animal Health Research Institute, Council of Agriculture Executive Yuan, Taipei, Taiwan

Introduction
Porcine circovirus type 2 associated diseases (PCVAD) has become one of the most concerned diseases in swine industry (4). Though the novel PCV2 vaccines have shown good protection for PCVAD control, implementation of a strict biosecurity and enhancement of host innate immunity are also very important in PCVAD control (2, 3). Sugar cane extract (SCE), a natural product, has displayed immunostimulating effects on porcine leukocyte functions (1). Therefore, this study was aimed to evaluate the effects of SCE and management improvement on PCV2 and/or PRRSV infection.

Materials and methods
Experimental design: The study was conducted on two separated experiments. In experiment I, the experimental pigs were weaned at 18 days old and moved into an experimentally controlled house to avoid PRRSV infection. In experiment II, the experimental pigs were weaned and raised at the original farm with PRRSV endemic until 8 weeks old for PRRSV natural exposure and then moved into experimental house. Experimental pigs were grouped and treated as indicated in table 1.

Table 1 Grouping and treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>SCE⁺</th>
<th>PCV2 chal.⁰</th>
<th>PRRSV inf.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp I NEG-1</td>
<td>4</td>
<td>No</td>
<td>No</td>
<td>0/4</td>
</tr>
<tr>
<td>POS-1</td>
<td>6</td>
<td>No</td>
<td>Yes</td>
<td>0/6</td>
</tr>
<tr>
<td>Exp II NEG-2</td>
<td>3</td>
<td>No</td>
<td>No</td>
<td>3/3</td>
</tr>
<tr>
<td>Pre-SCE</td>
<td>5</td>
<td>Yes²</td>
<td>Yes</td>
<td>5/5</td>
</tr>
<tr>
<td>Post-SCE</td>
<td>5</td>
<td>Yes²</td>
<td>Yes</td>
<td>5/5</td>
</tr>
<tr>
<td>POS-2</td>
<td>5</td>
<td>No</td>
<td>Yes</td>
<td>5/5</td>
</tr>
</tbody>
</table>

A, Provided by Shin Mitsui Sugar Co. Ltd., Japan; 500mg/kg.bw; B, 3 consecutive days every two weeks before PCV2 challenge; C, 3 consecutive days after PCV2 challenge; D, Pigs were challenged with PCV2 at 9 weeks of age.

Evaluation:
1. Clinical record: The body temperatures (BT) were recorded daily and fever was determined by the BT > 40°C. Body weight was weighted weekly.
2. Pulmonary impairments (PI, %) were scored depending on the area of bronchopneumonia (%), grossly and the severity of interstitial pneumonia (%), microscopically at the end of experiment, i.e. 4 weeks post PCV2 challenge.
3. PCV2 virus loads were determined by real time PCR.

Results
Pigs in group POS-1 that weaned early and segregated to the clean house had significantly lower PRRSV infection and ~21.5% lower MDWG after PCV2 challenge. In contrast, pigs in experiment II were all naturally exposed to PRRSV infection. After PCV2 challenge, all pigs were more seriously affected by PRRSV and PCV2 synergistic infection, with a decrease of 44.6% in MDWG (POS-2). However, SCE administration showed 9.4% to 20% improvement on the decreasing injury caused by PRRSV and PCV2 synergistic infections (table 2).

Table 2 Summary of the two experiment Results

<table>
<thead>
<tr>
<th>Group</th>
<th>MFD⁴</th>
<th>PI(%)⁵</th>
<th>PCV2(log10)</th>
<th>MDWG(gm)⁶</th>
<th>Affected(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEG-1</td>
<td>0</td>
<td>3.6</td>
<td>-</td>
<td>0.65</td>
<td>0</td>
</tr>
<tr>
<td>POS-1</td>
<td>2.7</td>
<td>17.8</td>
<td>10.42</td>
<td>0.51</td>
<td>-21.5</td>
</tr>
<tr>
<td>NEG-2</td>
<td>0</td>
<td>4.3</td>
<td>-</td>
<td>0.61</td>
<td>-6.2</td>
</tr>
<tr>
<td>Pre-SCE</td>
<td>3.0</td>
<td>28.5</td>
<td>11.03</td>
<td>0.49</td>
<td>-24.6</td>
</tr>
<tr>
<td>Post-SCE</td>
<td>4.0</td>
<td>23.7</td>
<td>10.98</td>
<td>0.42</td>
<td>-35.2</td>
</tr>
<tr>
<td>POS-2</td>
<td>7.0</td>
<td>34.3</td>
<td>11.20</td>
<td>0.36</td>
<td>-44.6</td>
</tr>
</tbody>
</table>

A, MFD: mean fever day; B, pulmonary impairment (%); C, MDWG: mean daily weight gain

Discussion
Administration of SCE can decrease the injury, but may not avoid the effect of PRRSV and PCV2 synergistic infection. In contrast, early weanling and segregation system can efficiently decrease or control PRRSV infection and also decrease the effect of PRRSV and PCV2 synergistic infections.

References
First report of the incidence and distribution of PCV2 virus in Mexico

Francisco Robles¹ Ricardo Angulo² Jean Claude Chevez¹ Edgar Diaz²

¹. Boehringer Ingelheim Vetmedica S.A. de C.V., Guadalajara, JAL, Mexico; ². Boehringer Ingelheim Inc., St. Joseph, MO, USA

Introduction and Objective
Porcine circovirus type 2 (PCV-2) has been reported as the cause of PMWS and others syndromes (1,2,3). This virus was identified in Mexico during 2001 (4) and different reports show the current presence of PCV-2 in Mexico. Nevertheless, there is not enough information about the prevalence and distribution in Mexico, just some reports of antibodies’ presence (5). The main objective of this study was to establish the prevalence of PCV2 in different regions of Mexico using the PCR technique.

Materials and Methods
The study was conducted in 34 farms located in 10 different regions of Mexico. In 20 of these farms, the pigs showed clinical PMWS, according with the case definition (4). The sampling period was from January to September of 2006, collecting serum (n=3094), organs (n=122) plus lymph node, lung, ileum, and fecal swab samples (n=437.) The samples were pooled in accordance with the type of sample. There were 888 pools in total. The conduction of a PCR test amplified a fragment of 506 nucleotides of the ORF2.

Results
The number of samplings was 89 with 72 samplings equaling 80.89%, testing positive. The analysis showed that 33 of 34 farms, equaling 97.06%, were PCV2 PCR positive.

Table 1. Results of PCV2 analysis for farms and Mexican state

<table>
<thead>
<tr>
<th>STATE</th>
<th>No. farms analyzed</th>
<th>PCV-2 Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>COAHUILA</td>
<td>1</td>
<td>NEGATIVE</td>
</tr>
<tr>
<td>GUANAJUATO</td>
<td>4</td>
<td>POSITIVE</td>
</tr>
<tr>
<td>JALISCO</td>
<td>4</td>
<td>POSITIVE</td>
</tr>
<tr>
<td>MICHOACAN</td>
<td>1</td>
<td>POSITIVE</td>
</tr>
<tr>
<td>MONTERREY</td>
<td>1</td>
<td>POSITIVE</td>
</tr>
<tr>
<td>PUEBLA</td>
<td>7</td>
<td>POSITIVE</td>
</tr>
<tr>
<td>QUERETARO</td>
<td>3</td>
<td>POSITIVE</td>
</tr>
<tr>
<td>SONORA</td>
<td>4</td>
<td>POSITIVE</td>
</tr>
<tr>
<td>VERACRUZ</td>
<td>1</td>
<td>POSITIVE</td>
</tr>
<tr>
<td>YUCATAN</td>
<td>8</td>
<td>POSITIVE</td>
</tr>
</tbody>
</table>

The analysis for each Mexican state shows that 9 out of 10 states are positive; this represents the 90% of positives. The only negative state was Coahuila. Nevertheless, for this state only one farm (3 samples) was subject to analysis.

Table 2 shows the results for each type of sample. In this case, the samples showing more findings that are positive were rectal swabs with 56 %, and the ones showing fewer positives were semen samples with less than 10%.

Table 2. Results of PCV2 analysis from different samples

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>NO. SAMPLES</th>
<th>NO. POOLS</th>
<th>NO. POSITIVES</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>SERUM</td>
<td>3094</td>
<td>682</td>
<td>268</td>
<td>39.3</td>
</tr>
<tr>
<td>TISSUE</td>
<td>122</td>
<td>110</td>
<td>43</td>
<td>39</td>
</tr>
<tr>
<td>SEMEN</td>
<td>22</td>
<td>11</td>
<td>1</td>
<td>9.01</td>
</tr>
<tr>
<td>RECTAL SWABS</td>
<td>437</td>
<td>85</td>
<td>48</td>
<td>56.47</td>
</tr>
</tbody>
</table>

Discussion
PCR is a sensitive diagnostic method widely used for pathogen detection, including PCV2. Since PMWS was described in Canada (1997), PCV2 has been isolated from different samples in Germany, France, Spain, Korea, Japan and other important pig-producing countries (3.) In America, there are reports of the presence of PCV2 in United States, Canada and Brazil, but in Mexico there is only one report of the antibodies’ presence and few reported cases have been isolated. Our results are the first report of the incidence and distribution of PCV2 in Mexico’s commercial farms (using the PCR method), and show that PCV2 virus is distributed throughout all of Mexico’s territory. In this research there was no difference between tissue samples and blood samples. The best samples for diagnostic purposes were found to be rectal swabs (56 per cent positives) and the worst were semen (almost 10 per cent positives). Nevertheless all the different sample types analyzed showed positive results.

The percentage of positives found during this investigation is similar at other reports from around the world.

It is very important to research the sequences of the Mexican PCV2 virus in order to determine the homology with other PCV2 virus distributed around the world.

References
Histopathologic findings of Porcine Multisystemic Wasting Syndrome (PMWS) with non suppurative myocarditis as a cause of sudden death in two pig farms

Elias J. Sogbe1 Carmen T. Diaz1 Jean Cano1 Vitelio Utrera1 Elias Ascanio1 Miguel E. Sogbe1 Andres Boulanger2 Susan V. Del Castillo1
1. Universidad Central de Venezuela, Maracay, Venezuela; 2. AABL Veterinaria C.A., Valencia, Venezuela

Introduction
In recent years Post Weaning Multisystemic Wasting Syndrome (PMWS) has become of significant concern in the swine industry in many countries, particularly Canada, United States of America, Europe, Asia and South America. The syndrome affects pigs mainly between 6 and 14 weeks of age. It is characterized by wasting or unthriftiness, dyspnea, jaundice and enlarged lymph nodes. The syndrome was been associated with myocarditis finding in aborted or stillborn piglets due to PCV2, it was also observed in 8-day-old piglet with PCV2 association (1) The present study was performed in piglets located in 2 porcine farms from Central State of Venezuela (Aragua/Carabobo).

The first description of the syndrome in Venezuela was in 2003 (4,5). Therefore in this report we focus on describe the heart histopathological findings in young pigs naturally infected in two Venezuelan pigs farms with sudden death piglets, both farms were free from classical swine fever, Aujeskys disease, Porcine reproductive respiratory syndrome and encephalomyocarditis

Material and Methods
The present study was performed in 20 piglets of 6 to 10 weeks of age from 2 pig farms placed at Central States of Venezuela (Aragua/Carabobo). All piglets showed signs of wasting, pallor, dyspnea, diarrhea and enlarged superficial inguinal lymph nodes and has history of sudden and unexplained death.

Each pig was necropsed and tissue samples were collected for the histopathological evaluation, fixed in Buffered formalin 10%, processed by rutinary methods, and stained with Hematoxilin-Eosin.

In situ hybridization studies (IHS) were performed on those specimens with histological evidence of PCV2 (heart included) as described by Segalés et al (3)

Results and Discussion
Clinical aspects: All the evaluated piglets (20) showed signs of wasting, pallor, respiratory distress, diarrhea, and sudden death.

At necropsy there was evidence of pneumonia with non-collapsible lungs as well as inguinal, mesenteric and tracheobronchial lymph nodes enlargement, heart showed focal to locally extensive hemorrhagic areas in myocardium with white spots areas.

The histopathology study showed lymphoid depletion, with loss of follicles architecture in lymph nodes, spleen, and Peyers patches, histiocytic infiltration, basophilic cytoplasmatic inclusions in histiocitic cells, and syncytial cells. These findings described were suggestive of PMWS. (3). The heart study showed focal to locally extensive areas of myocarditis and fibrosis. Other areas showed degeneration of myocardium and infiltration of macrophages and lymphocytes in the heart (Fig.1). In this areas cross striation in cardiomyocytes have disappeared and necroses foci were seen and could explain heart failure and sudden death (Fig 2)

The sample tissues specimen processed by a technique of IHS (Fig.3) revealed the presence of PCV2 in affected lymphoid tissue and heart and confirmed the diagnosis of PMWS.

Conclusions
The results confirmed the existence of PCV2 associated with nonsuppurative myocarditis as an important cause of sudden heart disease of young piglets in Venezuela

Microographies
Fig. 1

Fig.2

References
Introduction

Porcine circovirus 2 (PCV2) is associated with Postweaning Multisystemic Wasting Syndrome (PMWS) and other Porcine Circovirus Associated Diseases (PCVAD), but also is frequently isolated from healthy swine. PCVAD threatened the swine industry in Europe and later North America for about 15 years. In North America in 2006 efficacious PCV2 vaccines was introduced. However, little research to answer basic questions on the disease has been done since 2006.

Molecular biologists warn that this lack of understanding weakens our ability to respond to new outbreaks. We need to increase our understanding of PCVAD should current control strategies fail.

Our objectives were to retrospectively: 1. Determine the National Prevalence of PMWS and 2. the National Weaned Pig Mortality from PMWS, 3. Determine the Prevalence of PCV2 (Antibodies and PCR) and 4. Develop National Estimates for the impact of farm and animal level factors on incidence of PMWS.

Materials and Methods

Questionnaire data and blood samples were collected as part of the NAHMS Swine 2006 study. Questionnaires administered during personal interviews with farm personnel were used to gather data on housing, health management and health status. Blood samples were collected from up to 35 grower/finisher market pigs (20-32 weeks old) on 185 farms in 16 states. A new ELISA (n=6,046) was used to test for the presence of PCV2 antibodies and a new PCR (n=4,147) was also used to test whether PCV2 DNA was present in the sera of these pigs. In a separate study, these tests' accuracy was determined using Bayesian analysis. For the ELISA, the Sensitivity was up to 90% while that of the PCR was up to 92%.

Results

Nationally, approximately one in three sites with weaned market pigs reported PMWS during the 12 months prior to the interview, ranging from 29.7% of small sites (<2,000 head) to 59.9% (>4,999 head) of large sites. On sites that reported PCVAD in weaned market pigs, 15.4% of these pigs were affected and again, larger sites had more pigs affected (19.8% vs. 7.7%). Out of 6,046 samples 78.8% were positive for PCV2 antibodies and out of 4,147 samples 82.6% were positive for PCV2 DNA. Over 80% of samples tested using both tests were positive for antibodies and DNA. All but two farms had one or more samples positive for PCV2 antibodies and all but one for PCV2 DNA.

We used a weighted logistic regression that generates estimates that can be directly extrapolated back to the population (>93% of the farms nationally with 100 or more pigs) rather than just the sample it comes from. When farms had one respiratory disease (such as PRRS) the odds of PMWS being present went up almost 14 times compared to when no respiratory disease was present. If there were two or more respiratory diseases the odds went up over 30 times.

Discussion

Prior to the use of vaccines, more large farms than small farms had PMWS-affecting more animals. PCV2 elicits a humoral immune response yet fails to clear the virus for some unknown reason. Anecdotally, it was believed that PCV2 was ubiquitous but there has not been a study of this magnitude to give more accurate estimates.

Should the vaccines available now begin to fail to control PMWS or more broadly PCVAD, then we must return our efforts to reducing the impact of these respiratory pathogens modeled such as PRRS.

References

Porcine circovirus 2 as a causative agent for severe respiratory signs and polyserositis on a Specific Pathogen Free fattening farm

Victor Geurts¹ Chris Schouten² Antoine L.M. Cruijsen¹ Mark W. de Groot¹ Mieke P. Vrijenhoek¹
¹. Intervet Nederland BV, Boxmeer, Netherlands; ². Veterinarian Practice, Heeswijk-Dinther, Netherlands

Introduction

The clinical expression of PCVAD embraces a complex of signs including those related to the respiratory system. The authors are not aware of any reports relating polyserositis directly to PCV2 infections. Experimental PCV2 infections in Specific Pathogen Free (SPF) pigs has led to severe respiratory signs with high mortality including lung edema, hyperemia of the lungs, icterus, lymphadenopathy and edema of the mesentery (1). In 2009 on 2 locations SPF fattening pigs from one origin suffered from severe respiratory symptoms (dyspnea, coughing), high fever and a high level of mortality in spite of vaccination at 4 and 6 weeks of age against Haemophilus.parasuis (Porcilis®Glässer). Necropsy revealed polyserositis with negative bacteriological results, hyperemia of the lung, and interstitial lung edema. Organs were highly positive for PCV2 by IHC. Paired serological investigation of acutely sick pigs, only revealed seroconversion to PCV2. This strongly suggests that the clinical signs were related to PCV 2 infections in a similar situation as described in experimentally infected SPF pigs by Gauger et al(1). A PCV-vaccination trial was set up in order to help the farm and to explore the relationship between the signs observed and PCV2 infections. After this trial, all new piglets that entered the fattening units were vaccinated.

Materials and Methods

In a batch of 350 piglets, 197 were vaccinated with a single dose of Porcilis® PCV at 10 weeks of age, on their arrival in the fattening unit. The rest were left unvaccinated. Vaccinated and unvaccinated pigs were housed site by site in three different units. Mortality was recorded and in each trial group serum of 10 pigs was sampled at 10-, 14-, 18- and 22-weeks of age. Sera were tested on antibodies against PCV2 (Symbiotics), PRRS(Idexx), H. parasuis (Biovet), M. hyopneumoniae (Idexx) and the 42 kD outer membrane protein of A. pleuropneumoniae (2). Sera were also tested on PCV2 virusload by qPCR. Average daily intake (ADI), feed conversion rate (FCR) and average daily gain (ADG) during 2 months after the start of the vaccination was compared with the results of not vaccinated pigs during 2 months before the trial.

Results

All samples were negative against PRRS and M. hyopneumoniae. Also A. pleuropneumoniae titers stayed at low levels in both groups. Some sera of the 10- and 14-week old fatteners were positive against H. parasuis in both groups. These positive results were likely due to the early vaccination because all 22-week old fatteners became negative.

Conclusion and Discussion

The clinical signs (respiratory distress and polyserositis) on this farm were likely caused by PCV2, no other causative agent was found in the diseased pigs and vaccination against PCV was successful in preventing the disease. Pigs vaccinated with Porcilis® PCV had a reduced mortality, a clear humoral response to vaccination and the amount of PCV2 virus positive pigs was reduced. The historical data show a clear improvement in ADG, ADI and FCR after starting the vaccination. The respiratory signs in this clinical field trial with SPF pigs were similar to those of experimentally infected SPF pigs after PCV2 challenge (1). The authors are not aware of any reports relating polyserositis to PCV2 infection. A possible explanation for this feature could be a co-infection with Mycoplasma hyorhinis (3, 4), an opportunist which is capable of causing polyserositis. Its involvement may be triggered by the immune suppression caused by PCV 2 infection. The practical implication of the results of both this and the Gauger trial is that PCV 2 can be the cause of acute respiratory signs with high fever and high mortality levels.

References

1. Gauger P. C et al. 2006, Proc. OR.01.28 19th IPVS Copenhagen
Experimental co-inoculation with porcine circovirus type 2 (PCV2) and Mycoplasma hyopneumoniae in conventional pigs

Marina Sibila; Maria Fort; Miquel Nofrarias; Anna Pérez de Rozas; Ivan Galindo-Cardiel; Enric Mateu; Joaquim Segales
Centre de Recerca en Sanitat Animal (CReSA), Bellaterra, Spain

Introduction
Porcine circovirus type 2 (PCV2) is the etiologic agent of postweaning multisystemic wasting syndrome (PMWS) in pigs. However, PCV2 co-infection with some viral and bacterial agents may potentiate PMWS development (1). Particularly, a previous infection with Mycoplasma hyopneumoniae (Mh) has been shown able to trigger PMWS in subsequently PCV2 infected pigs (2). The objective of this work was to study the effect of concurrent PCV2 and MH co-inoculation in conventional piglets.

Material and Methods
Thirty six 6-week-old male conventional piglets were divided into 4 groups: control (n=6, receiving 2 ml of PBS); PCV2 (n=6, intranasally challenged with 1 ml of 105 TCID50 PCV2b per nostril); MH (n=12, intratracheally inoculated with 5 ml of MH 107 CCU on two consecutive days); and PCV2+MH (n=12, same inoculum conditions as above). Clinical signs, rectal temperature and body weight were recorded during the study period. Blood samples, faecal and nasal swabs were collected on 0, 7, 14 and 21 dpi. At necropsy (21 dpi), tracheal and bronchial swabs were also taken. Extension of cranio-ventral pulmonary consolidation lesions (CVPC) was assessed (3). Moreover, lymphoid tissues and lungs were collected and fixed in 10% buffered formalin. Sera were examined for PCV2 (total and neutralising (4)) and MH antibodies. DNA extracted from serum samples, nasal and faecal swabs were analyzed by a PCV2 quantitative PCR (Q-PCR) (5). PCV2 in situ hybridization (ISH) from lymphoid tissues and lung was performed (6). MH DNA from nasal, tracheal and bronchial swabs was analyzed by Q-PCR.

Results
PCV2 infection remained subclinical although mild PMWS-like microscopic lesions were observed in two PCV2 pigs and in one PCV2+MH animal. PCV2 was detected by ISH in 3 PCV2 and in 4 PCV2+MH challenged pigs. No significant differences on mean body weight and rectal temperature were observed among the 4 groups. Total antibody and NA titres were similar in both PCV2-inoculated groups. Number of PCV2 Q-PCR positive pigs in serum was higher but not statistically significant in all samplings in PCV2+MH than in PCV2 inoculated pigs. No significant differences in viral loads on different sampling days and duration of viremia were detected among PCV2 inoculated groups.

Coughing was observed in 3 piglets from the MH group and in 6 PCV2+MH pigs but it was absent in non-MH inoculated pigs. Proportion of MH Q-PCR positive pigs and mean bacterial DNA load in nasal cavity were similar in MH and PCV2+MH groups. On the contrary, mean MH DNA load at bronchial and tracheal swabs was higher in the PCV2+MH group compared to the MH one. No significant differences on percentage of MH seropositive pigs among MH inoculated groups were observed. Eight pigs from MH group and nine from PCV2+MH group showed CVPC. No significant differences in mean lung scoring between MH group and PCV2+MH group were observed.

Discussion
In a previous study, MH inoculation two weeks before PCV2 challenge resulted in an increase of PCV2-associated lesions severity, amount of PCV2 antigen and incidence of PMWS in pigs (2). In the present study, the concurrent inoculation of MH and PCV2 did not produce that synergic outcome. These divergent results may be explained by the different timing of infection but also by the source of the animals used. In the present study, the inoculated animals were seropositive to MH- and PCV2 while in the previous one (2) the challenged animals were seronegative against both pathogens. Taking into account that infection with both agents was successful, the present study suggests that timing and initial pig serological status may be the most important issues for the final outcome of the co-infection.

Acknowledgements
This work is based on data generated in a study commissioned by Intervet/Schering-Plough Animal Health. Part of these results was presented at a satellite symposium held at the IPVS 2008, Durban (South Africa) organized by this company.

References
High mortalities in the nursery, growing and finishing pigs. Risk assessment in Colombian pig herds

Carlos A. Diaz2 María N. Rodríguez1 Gloria Ramirez2 Jairo Jaime2 Victor Vera2 Jose D. Mogollón2

Introduction
Porcine Circovirus Associated Diseases (PCVD) have been associated with high mortality rates but PCV2 infection itself hasn’t been proved to be a risk factor for developing them (1, 2, 3). Some management practices, most be triggering the disease once PCV2 infects the pig. The main objective in this study was to determine what kind of swine industry practices and/or production indicators are associated with extremely high mortalities rates in Colombian swine herds.

Materials and Methods
Sample size was calculated in 70 herds considering a 95% confidence level, 10% precision and 50% prevalence. Herds were randomly selected from 163 farms located in the three major swine rearing areas in Colombia, with 200 or more sows that represent the 74% of national inventory. An epidemiological survey was applied, and extremely high mortality in the nursery and finishing was defined as mean plus 2 standard deviation of the sample without extreme values when calculating it. 43 serum samples were collected per production cycle and analyzed for PCV2 antibodies (SERELISA® PCV2 SYNBIOITICS). Samples of death animals were collected when present and analyzed for PCVD lesions and PCV2 antigen was identified by Immunohistochemistry (IHQ) within compatible lesions.

Statistical analysis was performed using STATA 9 ®. The null hypothesis was that exposure to any of the variables included in the survey do not modifies the risk of developing extremely high mortality rates. Association between high mortalities and exposure variables were assessed through a logistic regression model estimating relative risk adjusting it for variables like age (p=0.026). Mean mortality rate in the nursery was estimated in 1.41% SD 0.74 and 2.2% in the finishing pigs SD 1.45. The risk to have extremely high mortalities in the nursery was estimated to be 17.5 (IC 95% 1.8, 168.2; p=0.01) higher when farms don’t use shower baths at the entry to the nursery if the polyserositis (OR 6.1, IC95% 1.06-35.3; p=0.43) and low consumption (OR 25.3 IC95% 2.3 – 274.0; p=0.008) effect was controlled in the logistic model; all other variables were dropped off the model because they lost significance when the effect of this three was controlled. All of the variables included in the second model lost significance when the effect of low consumption was controlled in finishing pigs; it was estimated that finishing pigs have a risk 18.8 times higher to develop high mortality if low consumption was reported (IC95% 1.7 – 203.2; p=0.016). PCVD has been diagnosed and PCV2 has been identified within lesions in some farms but not all result were available at the time of this preliminary report. PCV2 exposure was determined in all farms evaluated and vaccination didn’t modify the risk of developing high mortality rates.

Discussion
PCV2 exposure itself was discarded to be associated with high mortality rates in the nursery and growing to finishing in farms with more than 200 sows in Colombia. Poor management practices could make possible that other swine pathogens, including PCV2, alter pig’s health status in such a way that high mortalities appeared. The difference between proportions in positive animals to PCV2 during the nursery and finishing period may be related to high mortalities in certain ages.

References
Growth depression after severe outbreak of porcine circovirus associated disease (PCVAD) in the finishing unit: case history

Piotr Kneblewski; Krzysztof Wilczynski; Pawel Karbowiak
Vet-Com, Olsztyn, Poland

Introduction
The first reports about confirmed PCV2 in Polish swine herds date back to 2000 (1,2). The aim of the investigation was to determine the impact of outbreak of severe porcine circovirus associated disease (PCVAD) on the performance in the finishing unit.

Materials and Methods
The investigation was conducted in a fattening farm located in the western part of Poland. The farm consisted of three barns for 2000 finishers with pens for 30-120 animals. Pigs for this study were sourced from a high health farm without clinical signs of PCVAD. They were not vaccinated against PCV2. A total of 1600 pigs were moved to the finisher farm at 40 kg of weight. The factors which aggravate problems were high temperature during transport and the quality of the feed in the beginning, which caused outbreak of watery, yellowish or grey diarrhea which affected up to 50% of the animals. After two weeks persistent cough appeared in 30-35% of pigs and the first cases of death were recorded. Numerous symptoms of PMWS and the first PDNS cases occurred. Weekly mortality increased to 5-10 pigs. Post mortem examination showed cachexia, paleness of the skin, reddish or blackish foci of cutaneous necrosis located mainly in the area of rump, neck and ears, enlargement of inquinal and mesenteric lymph nodes, various inflammation of digestive and respiratory system. Three sick pigs were culled. Their lymph nodes, samples of ileum, cecum, spiral colon and kidneys were analysed microscopically and tested by in situ hybridisation for PCV2(1,2). Additionally intestine samples were examined by PCR for Lawsonia intracellularis and Brachyspira hyodysenteriae. PMWS typical histopathological lesions and PCV2 DNA were detected in the submitted material. The result for Lawsonia and Brachyspira was negative. After two weeks with many cases of diarrhea and coughing treatment was started with Tiamulin 45% (tiamulin 8mg/kg for 6 days) and then Soludox 50% (doxycyclin 10mg/kg for 6 days) was administered in the drinking water. Such therapy only stopped the progress of disease for two weeks. After this time treatment had to be repeated. Moreover every sick pig was put in hospital pens. All facilities were cleaned and disinfected every day. Additionally all animals in hospital pens (300 pigs at the peak of disease) got TiaClor (CTC and Tiamulin) and Paracetam(NSAID) in the feed for 21 days. The weight of the pigs was recorded at introduction to the finishing unit and at slaughter. Average daily weight gain (ADWG) was calculated and number of days to slaughter were recorded. Performance of pigs affected with PCVAD was compared with pigs non-affected with PCVAD. Group A consisted of healthy pigs without any clinical symptoms of PCVAD and group B consisted of pigs with PCVAD.

Results
During the fattening period clinical signs of PCVAD infection occurred in 82% of the pigs (Group B). Only about 13% were not affected (Group A). Total mortality rate was 4.8%. The ADWG of pigs with PCVAD (Group B) was reduced compared to non affected (Group A) by 244g/day. Number of days to slaughter was extended by 21 days for group B compared to Group A (Table 1).

<table>
<thead>
<tr>
<th>Number of pigs</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average weight at the beginning (kg)</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>ADWG (g)</td>
<td>952</td>
<td>708</td>
</tr>
<tr>
<td>Number of days to slaughter</td>
<td>75</td>
<td>96</td>
</tr>
</tbody>
</table>

Discussion
Outbreak of PCVAD infection had serious financial consequences, reduction of DWG by 244g/day and extension of feeding days by 21 for pigs with PCVAD and the loss was estimated at 35,200 USD or about 27 USD per pig. Had these treatments with antimicrobials, isolation of affected pigs in hospital pens, cleaning and disinfection of pens not been carried out losses would have been higher.

References
Effect of co-infection of porcine circovirus type 2 and porcine reproductive and respiratory syndrome virus on porcine alveolar macrophages

Fei Song1 Qiqai He1 Weidong Chai1 Wentao Li1 Catherine Charreyre2
1. College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, China; 2. Merial Limited, Lyon, France

Introduction
Porcine reproductive and respiratory syndrome virus (PRRSV) and Porcine circovirus type 2 (PCV2) are currently a major concern for the swine industry worldwide. A lot of field surveys have indicated that dual PRRSV and PCV2 infection is common in pigs (3, 4). However, whether the immunosupression, in particular in alveolar macrophages, is stronger compared with the singular infection is still unknown.

Materials and Methods
Porcine alveolar macrophages (PAM) were isolated from lungs of 5-week-old piglets as Chang (2005) description. For subsequent experiments, PAMs were seeded at 24-well plates and infected with PCV2, PRRSV, PCV2+PRRSV (simultaneously inoculation) and PCV2 followed by PRRSV infection at 6hr later. The uninfected PAMs served as blank control. The supernatant and pellet of infected cells were collected at 0, 12, 24, 48, 60hpi.

We used quantitative real time PCR and indirect immunofluorescence assay (IFA) to analyze the adhesion and replication of PCV2 on PAMS, the cytopathic effect (CPE) and livability of PAMs were monitored and calculated by Typan Blue Staining Cell Viability Assay Kit, and mRNA levels of the cytokines (TNF-α, IFN-γ, IL-8) in PAMs were detected by semi-quantitative real-time PCR.

Results
The infection of PAMs with PRRSV neither increased the adhesion nor the replication of PCV2, but the distribution of PCV2 was changed in PAMs. PCV2 could not replicated nor induced CPE in infected PAMs, whereas the co-infected with two viruses could induce the cell death compared to single virus infected group. The expression of TNF-α, IL-8 in PAMS induced by co-infected group (different orders) were found to significantly increase and the increase was stronger than singular virus infected groups(p<0.001). However the co-infected group inhibited the expression level of IFN-γ compared to virus infected alone (p<0.001).

Discussion
The object of our work was to study the effect of PRRSV and PCV2 co-infection on PAMS. The results showed that no replication of PCV2 in PAMs was observed and this phenomenon was not associated with the present of PRRSV or not, PCV2 probably evade the immune systems through low-dose infection. As to the difference of PCV2 distribution between co-infected groups and single virus infection, more evidence is needed to explain this consequence. Besides, the addition of PRRSV to PCV2 caused more severe cell death and increased the expression of TNF-α, IL-8 than single virus infection, the results indicated co-infection may cause more serious pulmonary pathological lesions. IFN-γ is the broad-spectrum anti-virus agent, the stronger inhibition of expression caused by co-infected groups indicated PCV2 and PRRSV co-infection caused more severe immune suppression than infection with PCV2 or PRRSV alone.

Acknowledgement
The study was granted by Merial Limited.

References
PDNS was not a homogenous entity in the Swiss PMWS epizooty between 2003 and 2006

Sandra Welti 1,2 Titus Sydler 1 Esther Buergi 2 Enrico Brugnera 3, 2 Xaver Sidler 2

1. Institute of Veterinary Pathology, Vetsuisse Faculty, Zurich, Switzerland; 2. Division of Farm Animals, University of Zurich, Veterinary Faculty, Zurich, Switzerland; 3. Institute of Veterinary Pathology, University of Zurich, Vetsuisse Faculty, Zurich, Switzerland

Introduction

Porcine circovirus type 2 (PCV2) is the causative agent for post-weaning multisystemic wasting syndrome (PMWS) and is considered to be one of the most important viral pathogens of pigs in many countries. PCV2 has also been associated with additional diseases, referred to as PCV associated diseases (PCVAD) which include PMWS, some types of pneumonia, enteritis, reproductive failures and another distinct disease condition, the porcine dermatitis and nephropathy syndrome (PDNS). In Switzerland a PMWS epizooty commenced at the end of 2003 (1). We attempted to reconstruct this epizooty and the retrospectively reevaluated PDNS cases are presented here.

Materials and Methods

The Institute of Veterinary Pathology, Zürich, is the only laboratory in Switzerland which diagnoses PCV2 infections by immunohistochemistry (IHC) with the monoclonal antibody F217 according to the rules of the sixth framework program (2). A retrospective investigation of all available cases from our archive and the archives of other Swiss laboratories between 2003-2006 were used to elucidate the Swiss PMWS epizooty, which began at the end of 2003. In this material only 14 of 538 declared PCV2 infected animals were IHC negative after reevaluation. 131 cases had a low PCV2 antigen load in the lymphatic tissues and 383 moderate to high. For this communication we selected the cases where PDNS was diagnosed using the “typical” glomerulonephritis as the diagnostic criterion.

Results

PDNS was diagnosed in 43 out of 538 animals. 21 PDNS cases had only minimal PCV2 antigen in lymphatic tissues and 22 had a moderate to high PCV2 antigen load. The PMWS animals were, as expected, significantly younger than those with PDNS. The PDNS pigs with low PCV2 antigen load in the lymphatic tissues were the same age as those with moderate to high antigen load (Ø 17 weeks) but they had significantly higher body weights (Ø 44.1 kg, n=19 with available data) than those with moderate to severe PCV2 antigen load (Ø 29.8 kg, n=13 with available data) (Table 1). The cases with moderate to high PCV2 antigen content also showed depletion and histiocytic infiltrates in lymphatic tissues similar to PMWS.

Not all cases had skin lesions.

Table 1: Mean age and body weight of animals with PMWS and PDNS with low PCV2 content and PDNS with high PCV2 content (* P < 0.001)

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Age (weeks)</th>
<th>Body weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMWS</td>
<td>10.5 ± 3.8 * (n=167)</td>
<td>14.6 ± 8.8 (n=276)</td>
</tr>
<tr>
<td>PDNS: low PCV2 antigen content</td>
<td>17.0 ± 4.2* (n=9)</td>
<td><em><em>44.1 ± 20.7</em> (n=19)</em>*</td>
</tr>
<tr>
<td>PDNS: moderate to high PCV2 antigen content “mixed PDNS-PMWS”</td>
<td>17.5 ± 4.2 (n=6)</td>
<td><em><em>29.8 ± 11.9</em> (n=13)</em>*</td>
</tr>
</tbody>
</table>

Discussion

PDNS is suspected to be PCV2 induced (3), however we were not able to demonstrate any PCV2 antigen signal or DNA signal (by insitu hybridisation) within the glomerular lesions. PCV2 antigen signals were primarily in lymphatic tissues. However low PCV2 antigen load in lymphatic tissues is not defined as PCV2 disease because low quantities of PCV2 may be found in healthy animals.

Our set of PDNS cases was clearly divided into two subsets. One comprised PDNS animals with no to scarce PCV2 antigen and good body condition, the other subset suffered from PMWS (with high PCV2 antigen content and lesions in lymphatic tissue) followed by renal failure. We called these cases “mixed PDNS-PMWS”.

PDNS cases were infrequent in this investigation probably because the easily recognizable typical cases with dermatitis were not submitted for necropsy by the owners.

References

Distribution of Porcine Circovirus type 2 (PCV2), Torque teno virus (TTV) and a novel porcine boca-like virus (PBo-likeV) in pigs from postweaning multisystemic wasting syndrome (PMWS)-affected and non-PMWS-affected farms in archival UK samples

Michael J. McMenamy1 Irene McNair2 Catherine Duffy1 John McKillen2 Bernt Hjertner1 Catherine Mulholland2 Gordon Allan2
1. Queen’s University Belfast, Belfast, UK; 2. Agri-Food and Biosciences Institute Northern Ireland, Belfast, UK

Introduction
Porcine circovirus type 2 (PCV2) is acknowledged as the essential infectious agent of postweaning multisystemic wasting syndrome (PMWS)1. However, it has been noted that PMWS may not develop even though a pig has been infected with PCV21. It is possible that other agents may contribute to the development of PMWS. A possible potentiating co-factor in the development of PMWS by immunologic dysregulation is Torque teno virus genogroup 1 (g1-TTV), even though it is not considered a directly causative agent2. Another candidate which may play a role in PMWS development is a recently discovered porcine boca-like virus (PBo-likeV), identified by high-throughput sequencing in lymph nodes taken in a Swedish study from PMWS-affected pigs2. Here we investigate the distribution of PCV2, TTV and the new PBo-likeV in archival serum and tissue samples recovered from pigs from 5 UK farms in 2002.

Materials & Methods
37 sera samples were collected from pigs on all 5 UK farms, 2 of which were known to be non-PMWS-affected and the other 3 PMWS-affected. 21 pigs from 3 PMWS-affected farms also had tissue samples collected. Tissues included lung, liver, kidney, spleen and lymph nodes from each pig. Viral nucleic acids were extracted from sera and homogenised tissue samples. All sera and tissue samples were analysed by PCR for the presence of PCV2 using ORF2 specific primers1. Where possible, resultant amplicons were sequenced to ascertain the PCV2 genotype5. The samples were also assessed for the presence of g1-TTV4, g2-TTV and PBo-likeV2 using real-time PCR. g2-TTV was detected using a set of primers kindly provided by Dr. Annette Mankertz (Robert Koch-Institut, Berlin, Germany) (data not published).

Results
Tissue sample PCR results were pooled due to little variation in virus presence from tissue to tissue. The percentage distribution of viruses detected in the samples are detailed in Tables 1 and 2.

Discussion
The disparity in virus distribution evident in the sera samples could result from the PMWS-affected samples being comprised of sera from the 1st week of life which would limit pig exposure time to these viruses. However, non-PMWS-affected samples also included terminal bleeds at 12 weeks of age. It is apparent from the tissue samples that g2-TTV and PBo-likeV were more prevalent in PMWS-affected pigs. It is also of interest that PCV2 genotype 2a was present in sera samples from non-PMWS-affected farms although genotype 2b was exclusively found in tissues from PMWS-affected farms5. Further statistical analysis of these results is required to assess if incidence of a virus or specific genotype can be significantly related to disease status.

References
Serum endotoxin in pigs affected by wasting syndromes

Paolo Candotti; Sara Rota Nodari; Claudia Nassuato
IZSLER, Brescia, Italy

Introduction
Postweaning multisystemic wasting syndrome (PMWS) is one of the disease syndromes which have been collectively named porcine circovirus diseases (PCVD). PMWS mainly affects nursery and/or fattening pigs and wasting is considered the most representative clinical sign of this disease. It is considered a multifactorial disease in which other factors in addition to PCV2 are needed, in most cases, to trigger the clinical disease. These factors haven’t been clearly identified yet.

The aim of this study was to examine the levels of serum endotoxin in weaning piglets from farms with clinical symptoms of wasting compared to the levels in pigs from healthy farms.

Materials and Methods
Five (control farms) healthy farms and 13 (case farms) with a wasting syndrome that could be attributed to PMWS were selected for the study. Diagnosis of clinical PMWS and exclusion of other wasting agents was made through anamnesis, clinical findings and anatomo-pathological examination. Ten weaners were blood sampled in each farm (in control farms: animals perfectly healthy; in case farms: animals with a severe wasting syndrome). Blood samples were tested for lyopolisaccarides (LPS) with a Kinetic Cromogenic Assay.

Results
Mean and standard deviations (SD) of serum LPS (EU/ml) in the different farms are reported in table 1.

In figure 1 the graph of sensitivity-specificity and the cut off value is reported.

Discussion
The correlation between the presence of the disease and the level of LPS was statistically significant. The average LPS level in healthy animals was 0.34 EU/ml (CI 95%; 0.08-0.59) while in wasted animals 0.92 EU/ml (CI 95%; 0.76-1.08). In presence of wasting, LPS was on average 0.58 EU/ml higher (CI 95%; 0.28-0.88; p<0.001). The cut-off value identified for LPS was 0.44 EU/ml.

The limitation of these findings is that PMWS was not confirmed by histopathological examination of lymphoid tissues and detection of PCV2 in damaged tissues. However, these results could be useful in identifying triggering factors in animals affected by wasting syndromes that can’t be attributed to specific pathogens and are clinically attributed to PMWS.

References
Conventional sows inseminated with artificially PCV2 infected semen: I. In vivo results

Giuseppe Sarli1 Federico Morandi1 S. Panarese1 B. Bacci1 D. Ferrara1 Laura Fusaro1 M.L. Bacci1 G. Galeati1 M. Dottori2 P. Bonilauri2 D. Lelli3 Giorgio Leotti4 Thaïs Vila5 François Joisel5 F. Ostanello1
1. School of Veterinary Medicine, University of Bologna, Bologna, Italy; 2. IZSLER, Reggio Emilia, Italy; 3. IZSLER, Brescia, Italy; 4. Merial, Milano, Italy; 5. Merial S.A.S., Lyon, France

Introduction
Since 1999, field evidence of transplacental infection by PCV2 and reproductive failure has been reported (1). Several experimental studies were performed: trans-uterine inoculation of fetuses (2), oronasal inoculation of pregnant SPF (3) or conventional (4) sows, intra-uterine inoculation of SPF sows (5). The objective of this study was to evaluate the clinical and pathological consequences of PCV2 infection in conventional pigs by artificially infected semen.

Material and Methods
Nine prepubertal conventional pigs were randomly divided: 3 controls and 6 infected. Hormonal estrus synchronization was followed by artificial insemination (AI) with a single dose semen added with 10 ml of a PCV2 suspension. After ultrasonography at 29 day post-insemination (DPI), empty sows were euthanized at 30 DPI whilst pregnant ones between 52nd and 56th DPI. Cervix, nasal and rectal swabs, and blood samples were weekly collected from -2 DPI till the end of the experimental period. Serum samples were directly and indirectly tested for PCV2, PRRSV, PPV and ADV. Serum antibody titres were determined by testing serial dilutions of each serum, by competitive ELISAs (6, 7, 8). The protocol described by Olvera et al. (9) was employed for PCV2 real time-PCR. Serum progesterone levels were measured by radioimmunoassay (10).

Results
At the -2 DPI, none pig presented viremia for all tested virus. All animals showed a high anti-PCV2 antibody level at -2 DPI (range 1/1000-1/10000), but only one sow had a lower titre (1/100). Four out of 6 infected sows displayed viremia at 7, 21, 28 and 35 DPI (the subject with the lowest anti-PCV2 level kept showing positive blood results for two successive samplings at 21 and 35 DPI). This latter was also the only sow showing a positive swab (rectal, 35th DPI). The anti-PCV2 antibody proved a decline after 7 DPI followed by a plateau in controls, whereas in infected animals were recorded values higher than controls that declined only after 42 DPI (Figure 1).

Discussion
In the present study the assessment of a field situation employing conventional pigs and intrauterine PCV2 exposition was investigated. Viremia was recorded in 4 out of 6 infected animals, besides mean antibody titre higher only in exposed subjects. Three infected sows out of 6 were not pregnant, unlike controls were all pregnant. Moreover, it should be emphasized that only the infected sow with lower PCV2 antibodies titre at -2 DPI showed simultaneously viremia and fecal virus spread. Therefore, i) the PCV2 infection is possible in conventional sows by intrauterine exposition; ii) low antibody titres increase the probability of the infection; iii) PCV2 infection close to insemination reduces the pregnancy rate.

References
Occurrence of PCV2 and TTV genotypes from fetus and from nursery and finishing animals
Kledna C. P. Reis; Jose L. Santos; Walter V. Guimaraes; Lucas F. Santos; Mayka R. Henriques; Daniel L. Santos
Microvet, Vicosa, MG, Brazil

Introduction
Swine with Postweaning Multisystemic Wasting Syndrome (PMWS) under PCR analysis yields both Porcine Circovirus genotypes (PCV2a and PCV2b) as well as association with Torque Teno Virus (TTV), PRRSV, Parvovirus (PPV), Swine Influenza, Coronavirus and also E. coli, Streptococcus suis, M. hyopneumoniae, H. parasuis and A. pleuropneumoniae (1,2,3). This work was done to verify the occurrence of PCV2 and TTV genotypes in samples from swine with PMWS and possible other virus and bacteria co-infections.

Material and Methods
Eighteen samples from swine suspected of PMWS, collected during 2009 in Brazil, were analyzed. Ten samples were from fetus (group I) and eight from nursery/finishing animals (group II). Lymph nodes, lung/heart were investigated by PCR analyses for PCV2a, PCV2b, TTV1, TTV2 and PPV. The DNA extraction and the PCR analyses were done according to Kim et al. (2003). The secondary bacteria infection diagnosis was done by Microvet laboratory (Brazil).

Results
PCV2b was the most prevalent in both groups. Two samples from group I were positive for PCV2a/PCV2b but this co-infection was not detected in group II. In both groups the most common co-infection was PCV2b/TTV2 followed by PCV2b/PPV. Twenty percent of samples from group I and 50% from group II had TTV1/TTV2 co-infection (Table 1). In group I, 60% of the samples were co-infected with one bacterium, mainly, alpha hemolytic E. coli and in group II different species were detected (Table 2).

Table 1. PCR results for 18 samples from fetus and nursery/finishing animals

<table>
<thead>
<tr>
<th>PCR positive virus</th>
<th>Group I (fetus)</th>
<th>Group II (nursery/finishing)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV2b</td>
<td>8/10 (80%)</td>
<td>8/8 (100%)</td>
</tr>
<tr>
<td>PCV2a/PCV2b</td>
<td>2/10 (20%)</td>
<td>0/8 (0%)</td>
</tr>
<tr>
<td>PCV2b/PPV</td>
<td>3/10 (30%)</td>
<td>6/8 (75%)</td>
</tr>
<tr>
<td>PCV2b/TTV1</td>
<td>2/10 (20%)</td>
<td>4/8 (50%)</td>
</tr>
<tr>
<td>PCV2b/TTV2</td>
<td>9/10 (60%)</td>
<td>7/8 (87.5%)</td>
</tr>
<tr>
<td>TTV1/TTV2</td>
<td>2/10 (20%)</td>
<td>4/8 (50%)</td>
</tr>
</tbody>
</table>

Table 2. Bacterial species associated with PCV2 in 18 samples from fetus and nursery/finishing animals

<table>
<thead>
<tr>
<th>Groups</th>
<th>Bacterial secondary agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>E. coli (6/10); S. suis 1/10</td>
</tr>
<tr>
<td>Group II</td>
<td>E. coli (2/8); S. suis (2/8); M. hyorhinis (1/8); L. intracellulares (1/8)</td>
</tr>
</tbody>
</table>

Discussion
The viral and bacterial co-infections are common in PMWS (1,2,3). The occurrence of PCV2 and TTV genotype infections could be related to differences in pathogenesis or animal origin. According to Horlen et al (2007) both PCV2a and PCV2b genotypes were found in isolates from PMWS-affected pigs. In this study the presence of PCV2a and PCV2b in group I needs more investigation since they were absent in group II. The PCV2a could be an antecessor in the primary infection or related to abortion or fetus anomalies when in association with PPV and TTV2. The presence of TTV1 and TTV2 in both groups was high, essentially the TTV2. The analysis of infection sequence in animals with PMWS can give interesting clues for the role of the TTV during infection. Taira et al. (2009) using nested PCR found positive results for TTV1 and TTV2 in different organs form PMWS swine. More studies are required to determine if other endogenous or exogenous cofactors are involved in the pathogenesis of PCV2a and PCV2b. In conclusion it can be stated that PCV2b and PCV2b/TTV2 were frequently observed and co-infection with bacteria were common in swine with PMWS.

References
Association of PCV-2 with porcine respiratory disease complex (PRDC) in field infection from nine swine-producing Brazilian states

Juliana Amália F. Nascimento¹ Fábia S. Campos¹ Grazielle C. Galinari¹ Ernane F. Nascimento² Adrienny T. Costa³ Marcos B. Heinemann¹ Zélia Inês P. Lobato¹

1. Laboratório de Pesquisa em Virologia Animal, Escola de Veterinária, UFMG, Belo Horizonte, MG, Brazil; 2. Departamento de Clínica e Cirurgia Veterinárias, Escola de Veterinária, UFMG, Belo Horizonte, MG, Brazil; 3. Instituto de Pesquisas Veterinárias Especializadas Ltda (IPEVE), Belo Horizonte, MG, Brazil

Introduction
The porcine circovirus diseases (PCVD) rarely occur as a result of the infection from one infectious agent, like PCV-2, alone. Usually several pathogens work together with the PCV-2 to break down defenses leaving the respiratory tract susceptible to pathogenic organisms. The PCV-2 has been identified in association with lesions in swines with porcine respiratory disease complex (PRDC). The aim of this work was to investigate the presence and distribution of PRDC bacterial coinfections in swines with clinical signals of the PCVD among Brazilian states.

Materials and Methods
Samples from 150 swine lungs with clinical symptoms of PCVD were studied. The samples were originally from field infections collected in 9 different swine-producing Brazilian states and have been previously confirmed to be positive for PCV-2 by PCR. All samples were submitted to microbiological standard tests for bacteria isolation and identification. Histopathological evaluation was carried out in 71 of the 150 samples and microscopic lesions were correlated with presence or absence of associated bacteria.

Results
Forty two samples (28.0%) did not present any bacterial agent associated. In 108 (72%) samples at least one of the following agents was detected: P. multocida type A, P. multocida type D, B. bronchiseptica, H. parasuis, A. pleuropneumoniae, S. suis, E. coli. Sixty-seven samples (44.7%) presented one bacterial agent only, while 41 (27.3%) had more than one. The bacterial frequencies among the 67 samples were: 18/67 (26.9%) of S. suis, 7/67 (10.4%) of A. pleuropneumoniae, 22/67 (32.8%) of P. multocida type A, 6/67 (9.0%) of P. multocida type D, 3/67 (4.5%) of B. bronchiseptica, 7/67 (10.4%) of H. parasuis and 4/67 (6.0%) of E. coli. In the 41 samples with more than one of these bacteria, 24 different pathogens combinations were observed. The distribution of the bacteria in the Brazilian states studied showed diversity. The most prevalent bacteria associations were A. pleuropneumoniae and P. multocida type A; S. suis and P. multocida type A; S. suis and P. multocida type A and H. parasuis. Minas Gerais state showed the major prevalence of bacterial associations. The pulmonary histopathological lesions observed in samples were grouped as: (i) positive association with bacterial infections (47.89%), (ii) negative for bacterial association (52.11%). In the negative group, interstitial pneumonia with thickening of interalveolar walls and inflammatory cells in the alveoli were observed. It was also observed peribronchial and perivascular lymphohistiocytic infiltrates with syncytial cells. In the positive group, pulmonary lesions similar to those from the negative group were observed in 12 samples, while pulmonary lesions with characteristics of bacterial infection were seen in 22 samples.

Discussion
In the present study we observed a wide dispersion of pulmonary bacteria across different swine-producing farms from nine Brazilian states. Furthermore, the frequency of association between PCV-2 and PRDC bacteria was high, suggesting that the PCV-2 immunosuppression can facilitate these pathogens infection. The results confirm that the respiratory infections are widely spread out among Brazilian herds. Our data are in accordance with previous studies that indicated that pulmonary lesions associated with PRDC had prevalence taxes of the 19.7% to 75.7% in swine from different Brazilian states. The high number of different bacterial associations with PCV-2 found reinforce current literature data that demonstrated that the PRDC is a multifactorial disease. It is also important to observe that Minas Gerais is one of the major swine producer states and that its herds are distributed for different states which could explain the dispersion of PCV-2 infection with bacterial associate.

References
Superficial inguinal lymph node inv various PCVD conditions

John Carr
Portec Australia, Belmont, WA, Australia

Introduction
An increase in size of the superficial inguinal lymph node is described as a clinical sign of Post-weaning Multisystemic Wasting Syndrome (PMWS). However, examination of the physical properties of the superficial inguinal lymph node indicates that rather than enlargement, the lymph node becomes more prominent as the “flare fat”, around the lymph node, disappears with the loss of weight associated with PMWS and other Porcine Circovirus Diseases (PCVD). In Porcine Dermatitis and Nephropathy Syndrome (PDNS), there was a true enlargement of the superficial inguinal lymph node both in terms of length, width and depth.

It is essential, when describing pathology, to avoid terms like “enlargement” without providing physical dimensions.

Materials and Methods
Normal population: Superficial inguinal lymph nodes were collected from a slaughterhouse or a feed-trial in Western Australia from a variety of live-weight pigs. All the pigs were passed suitable for human consumption or had no gross pathology. The lymph nodes were measured for their length, width and depth. Western Australia was selected because the area is free of many viral pathogens that cause changes in lymph node sizes – particularly Porcine Reproductive and Respiratory Syndrome virus.

Number of animals in each normal group:
10 to 30 kg live weight: 107 pigs
40 to 60 kg live weight: 312 pigs
90 to 110 kg live weight: 125 pigs

Abnormal population: Superficial inguinal lymph nodes were collected from cases of PMWS, PCVAD and PDNS.

Porcine Circovirus Associated Disease (PCVAD) is defined as pigs from farms where there was a PCVAD score ≥1 but there was no clinical evidence of PMWS on the farm.

Count: PCVAD: 33 pigs; PMWS: 17 pigs; PDNS: 10 pigs

Approximate age of the pigs in each group and normal weight range for the age group
PCVAD: 8 to 10 weeks  22 to 30 kg
PMWS: 8 to 16 weeks  22 to 65 kg
PDNS: 16 to 20 weeks  65 to 95 kg

Results
Length of the superficial inguinal lymph node:

<table>
<thead>
<tr>
<th>Mean mm</th>
<th>Standard deviation</th>
<th>Range mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 to 30 kg</td>
<td>38</td>
<td>7.4</td>
</tr>
<tr>
<td>40 to 60 kg</td>
<td>56</td>
<td>8.9</td>
</tr>
<tr>
<td>90 to 110 kg</td>
<td>61</td>
<td>9.3</td>
</tr>
<tr>
<td>PCVAD</td>
<td>44</td>
<td>7.4</td>
</tr>
<tr>
<td>PMWS</td>
<td>44</td>
<td>8.7</td>
</tr>
<tr>
<td>PDNS</td>
<td>90</td>
<td>21.7</td>
</tr>
</tbody>
</table>

Discussion
The normal inguinal lymph node increases in size as the pig grows from a mean length of 38 mm in pigs less than 30 kg to 61 mm in the 110 kg finisher pig.

The superficial inguinal lymph node is quite large and is normally virtually invisible; covered by fat.

In cases of cachexia the ‘flare fat” disappears around the superficial inguinal lymph node, which then becomes clinically very apparent.

In cases of PCVAD and PMWS the lymph node is not enlarged, when compared to the normal pig. However, it becomes more prominent because the surrounding “flare fat” is lost.

The superficial inguinal lymph node is enlarged – in terms of length, breath and depth in cases of PDNS. P<0.001. As the tissues of the skin around the perineal region are infected in cases of PDNS, an enlargement of the draining lymph node is to be expected.

Please visit out website: www.portec.com.au
Acute Pulmonary Edema (APE), a New Porcine Circovirus Associated Disease??

Kara M. Theis¹ Steve Henry² Lisa Tokach²

1. University of Minnesota, Lino Lakes, MN, USA; 2. Abilene Animal Hospital, Abilene, KS, USA

Introduction

We are investigating an on-going case involving porcine circovirus (PCV) and PRRS that has striking clinical differences compared to outbreaks before the advent of circovirus vaccines. Historically clinical circovirus is seen in 8-20 week old pigs with lesions of depleted lymphoid tissue in many organs. Gross pathology is varied and the clinical course is chronic, debilitating and progressive. Implementation of PCV vaccine decreased morbidity and mortality dramatically. In our case the clinical presentation differs with acute death pigs in good body condition. The consistent gross lesion, severe interlobular pulmonary edema, is distinguishing. The affected animals have been vaccinated with a product and protocol that has proven highly effective in the past. This particular animal group is suffering from severe disease while cohort animals, raised on other sites, remain normal.

At 21 days of age weaned pigs from two separate PRRS negative breed-to-wean farms move to one of 20 separate growing facilities, either stand-alone nurseries or nursery-finishing sites. Pigs in 9 of the 20 sites remain PRRS negative through their growth period while PRRS infection and seroconversion frequently occurs at 8-10 weeks of age in the other eleven sites. APE associated losses occur in both PRRS positive and PRRS negative populations. The 20 sites are fed by six separate and non-affiliated feed mills. All grain used has been 2008 grain sources. The four affected sites are fed by three different mills. None of the affected sites are located near one another. All pigs were immunized at four and seven weeks of age with Circumvent PCV vaccine (Intervet Schering-Plough Animal Health) Immunization protocol has remained unchanged since 2007 and has been highly efficacious. Groups affected range from 7-14 weeks of age at onset of clinical signs. In severely affected lots mortality ranged from 13-21%. Predominantly the mortality is acute and associated with APE, however some chronic and debilitated animals developed in certain groups.

Materials and Methods

Fresh and fixed tissues were collected from seven pigs of seven weeks of age and weighing approximately 35 lbs. Three pigs were found dead the morning of necropsy. The four remaining pigs were selected as “clinically normal” controls based on clinical evaluation. None of the other pigs in this four room nursery were coughing. They were clinically normal and were highly active. Pigs were sedated with 1ml of pentobarbital via jugular venapuncture. Seventy mls of blood was collected into 10ml serum separator tubes via an intracardiac stick. Animals were then euthanized with 1.5 mls of pentobarbital and necropsied. Gross pathology, histopathology including IHC for Circovirus, differential and quantitative PCR for PCV and PRRS, IFA antibody titters for PCV, viral genome sequencing and compliance antibody differential sampling with baculovirus antibody testing was conducted.

Results and Discussion

On the basis of the viral genome sequencing the circovirus and PRRS viruses present are very similar to those historically known to this region and production flow. Compliance antibody evaluations exhibited responses to vaccination and several groups that exhibited disease appeared to have been well vaccinated based on compliance testing. Through IHC, histopathology and PCR testing circovirus was also demonstrated as the agent associated with the pulmonary pathology, including necrotizing vasculitis, which is not commonly seen with PCV2. The concentrations of circovirus detected in the tissue and the serum were considered extremely high in most cases with Ct <10 for many tissues and Ct < 20 for serum pools.

While interlobular edema is an occasional manifestation of circovirus the pulmonary edema is not. Noncardiogenic pulmonary edema is characterized by diffuse alveolar damage, increased capillary permeability and accumulation of protein rich fluid in the alveoli. The fluid accumulation ultimately results in decreasing oxygenation. This can be caused by either direct alveolar damage i.e. diffuse pulmonary infection or indirectly i.e. severe sepsis resulting from the over expression of the normal inflammatory response. Commonly all of this occurs within 24 hrs manifesting as respiratory failure and acute death a,b.

The consistent lesions of acute death, interlobular edema, necrotizing vasculitis pleural transudate accompanied by very high concentrations of virus in tissues appears clinically to be a new manifestation of PCV2b viremia. Several laboratory tests are still pending.

References

Introduction and Objectives
Porcine Circovirus type 2 (PCV2) is a worldwide endemic viral pathogen of swine, associated with Postweaning Multisystemic Wasting Syndrome (PMWS). PCV2 infection is essential to the development of PMWS, but other co-factors can influence the evolution of PCV2 infection (1). Between these co-factors there are co-pathogens. An enhancement of PCV2 viral load, severity of the lesions and incidence of PMWS have been highlighted when PCV2 co-exists with other swine pathogens like PRRSV or Mycoplasma hyopneumoniae (2,3). Through the diagnostic outcome of investigations, carried out between January 2007 and August 2009 at IZSLER-Brescia, the aim was to determine the prevalence of several viral and bacterial swine pathogens, frequently associated with PCV2 in PMWS cases.

Material and Methods
193 PMWS affected pigs, from 92 farms, were investigated for other swine pathogens. For diagnosis of PMWS was applied a protocol according to Opriessnig et al., 2007(1). For PRRSV was applied a reaction One-Step RT-PCR multiplex with specific primers, described by Persia et al., 2001(4). For SIV a RT-PCR according to the protocol described by Spackman et al., 2002(5). For Mycoplasma hyopneumoniae a PCR according to the protocol described by Stakenborg et al., 2006(6). For Lawsonia intracellularis a PCR as described by Jone et al., 1993(7). For Haemophilus parasuis was applied a PCR described by Angen et al., 2007(8). Isolation of bacterial agents was performed by routine culture procedures.

Results and Discussion
The viral and bacterial pathogens identified in PMWS affected pigs are reported in the table below.

The co-pathogens association is shown in Graphic 1.

References
A preliminary study of the effects of treating diarrhoeic pigs with oxytetracycline on shedding of Porcine Circovirus Type 2 and Lawsonia intracellularis

Patricia K. Holyoake1 Charlotte Hjulsager2 Lars E. Larsen2 Ken S. Pedersen3 Markku Johansen4
Helle Stege1 Beverley Orchard1 Marie Stahl1 Oystein Angen2 Jens P. Nielsen3

1. Wagga Wagga Agricultural Institute, Wagga Wagga, NSW, Australia; 2. National Veterinary Institute, Technical University of Denmark, Copenhagen, Denmark; 3. University of Copenhagen, Copenhagen, Denmark; 4. Danish Pig Research Centre, Copenhagen, Denmark

Introduction
There is pressure to minimize antibiotic use in food-producing animals due to the risks of selecting resistant strains of bacteria. In Denmark, pressure to reduce macrolide antibiotics has resulted in a tendency to increase the use of tetracyclines.1 Most antibiotics are used to treat intestinal infections of grower (>10 weeks) pigs, thought to be caused by Lawsonia intracellularis (L. intracellularis) and/or Porcine Circovirus Type 2 (PCV2). Our hypothesis was that individual treatment of diarrhoeic pigs for three days with an oxytetracycline antibiotic would reduce diarrhoea and the faecal shedding of L. intracellularis and/or PCV2.

Materials and Methods
Forty grower pigs were selected on each of six farms (total of 240 pigs) in Denmark for a case-control study. Each pig was ear-tagged and assessed for faecal consistency (normal, loose, fluid). Approximately half of the pigs had loose or fluid faeces and were classed as ‘diarrhoeic’. Faecal samples were collected from each pig and tested using real-time PCR specific for PCV2 and L. intracellularis.2,3 Half of the pigs in each group were randomly selected on Day 1 and injected for three consecutive days with oxytetracycline (Engemycin, Intervet). Pigs were re-assessed on Day 4, when repeat faecal samples were collected. The effect of oxytetracycline treatment on faecal PCV2 quantity (negative/low versus moderate/massive), and L. intracellularis bacterial quantity (negative/low versus moderate/massive) on Day 4 were analysed, with diarrhoea status (+/-), PCV2 quantity and L. intracellularis quantity on Day 1 included as fixed effects. All analyses were conducted using generalized linear mixed models, with pen and farm as random effects (Genstat 11th edition).

Results
Two farms had no pigs with moderate/massive PCV2 results in faeces. One farm had no pigs with a negative/low faecal PCV2 result. Two farms had pigs with no moderate/massive L. intracellularis results. There appeared to be within-pen clustering of PCV2 infection on Day 1, with all or no pigs in some pens being infected. This was also true for L. intracellularis on Day 4. Hence, pen was removed from subsequent analyses involving these variables. Pigs that had diarrhoea on Day 1 and had not been treated with oxytetracycline had 2.6 (95%CI 2, 3.2) times the odds of having diarrhoea on Day 4 than pigs that were treated (p<0.05). The farms where no pigs had moderate/massive quantities of PCV2 and/or L. intracellularis were excluded from analyses of associations between the two pathogens. Oxytetracycline treatment and PCV2 quantity on Day 1 did not affect PCV2 quantity on Day 4 (p>0.05). There were no significant associations between the quantities of L. intracellularis bacteria on Days 1 and 4 (p>0.05). However, treated pigs had 0.076 (95% CI 0.03, 0.19) times the odds of having moderate/massive quantities of L. intracellularis on Day 4 than non-treated pigs.

Discussion
These preliminary results suggest that oxytetracycline treatment of growing pigs did not affect the quantity of PCV2 shed in faeces. Treating pigs for three consecutive days with injectable oxytetracycline reduced the incidence of diarrhoea and the quantity of L. intracellularis shed in faeces, suggesting that this pathogen played a role in causing diarrhoea, and that the treatment was effective.

Acknowledgements
This project was supported by the University of Sydney, University of Copenhagen, Danish Pig Research Centre, Technical University of Denmark and Australian Pork Ltd. We thank the participating producers and veterinarians for their assistance.

References
1. Jensen VF. et al. (2008). “Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark.” ISSN 1600-2032
Presence of porcine circovirus type 2, Lawsonia intracellularis and Brachyspira hyodysenteriae in pigs with diarrhoea

Anna Szczotka; Jacek Zmudzki; Zygmunt Pejsak; Tomasz Stadejek
National Veterinary Research Institute, Pulawy, Poland

Introduction
The aim of the study was to analyze the presence of porcine circovirus type 2 (PCV2) in cases of antibiotic non-responsive diarrhoea and to evaluate the possible role of the virus in development of enteritis in pigs. For differential diagnosis identification of Lawsonia intracellularis (L. intracellularis) and Brachyspira hyodysenteriae (B. hyodysenteriae) was performed.

Materials and Methods
Internal organs and feces were collected from 76 pigs, 5 – 19 weeks old, from 50 farrow-to-finish farms, PMWS-positive or PMWS-suspected. Sections of lymph nodes and intestines (ileum, caecum and colon) were analyzed for presence of PCV2 DNA by in situ hybridization test (ISH) (2). They were also hematoxilin-eosin (HE) stained for standard histopathological examination. Additionally, fecal samples were tested for presence of B. hyodysenteriae and L. intracellularis by PCR (3).

Results
In samples from 37 pigs, 10 – 17 weeks old, large amounts of PCV2 DNA, typical for PMWS, were detected in lymph nodes by ISH. In this group, in samples from 19 pigs, PCV2 was also found in abundant amount in samples of ileum. The remaining 18 pigs, PCV2-positive in lymph nodes, were negative in ileum. In samples from only 1 animal lymph nodes were negative for PCV2 in ISH, but virus was detected in considerable amount in ileum. In HE stained sections of lymph nodes histopathological lesions characteristic for PMWS were identified. Similar lesions were observed in PCV2-positive samples of ileum. Seventy samples of feces were negative in PCR for B. hyodysenteriae and L. intracellularis. DNA of L. intracellularis was found in feces from 3 pigs and mixed infection caused by both bacteria was detected in 3 animals

Discussion
According to the obtained results in PMWS-affected pigs similar lesions could be observed both in lymph nodes and in ileum and they correlate with clinical outcome of disease. Also, it was found that presence of PCV2 in ileum could be correlated with diarrhoea in PMWS-free animal, which confirms that the emergence of this virus as an intestinal pathogen may represent a new phenomenon (1). In the animals negative for B. hyodysenteriae and L. intracellularis and PCV2 other causative agents of diarrhoea should be considered. Further investigation on larger number of samples needs to be performed to confirm the role of PCV2 as an etiological agent of diarrhoea in pigs.

Acknowledgements
This work was supported by the research grant N N308 075634 from Polish Ministry of Science and Higher Education

References
2. Stadejek T. et al. 2006, Medycyna Wet. 62, 297-301
Co-infection between porcine circovirus type 1 (PCV1) and genogroups 1 and 2 of porcine torque teno virus (TTV) in fetuses naturally infected by porcine circovirus type 2 (PCV2)


1. Virus and Prion Diseases Research Unit, NADC, ARS, USDA, Ames, IA, USA; 2. Embrapa Swine and Poultry Research Center, Animal Healthy Laboratory, Concordia, SC, Brazil; 3. Agroveterinarian Science Center - CAV – University of Santa Catarina State – UDESC, Lages, SC, Brazil; 4. College of Agronomy and Veterinary Medicine, University of Passo Fundo, Passo Fundo, RS, Brazil; 5. Oswaldo Cruz Institute, Rio de Janeiro, Brazil

Introduction
Porcine circovirus 2 (PCV2) associated diseases (PCVAD) includes reproductive failures (1). PCV2 DNA has been detected by nested-PCR in organs of fetuses naturally infected (8,5). In contrast PCV1 has not been associated with pathologies in pigs. Another DNA circular virus, porcine torque teno virus has also been detected in swine, with at least two genogroups, TTV1 and TTV2 (7). Recent studies have shown a possible role of TTV in increasing clinical signs and severity of lesions caused by PCV2 on piglets (3). The objective of this work is to study the co-infection of both porcine circular DNA viruses, TTV1, TTV2 and PCV1 in organs from fetuses naturally infected by PCV2, aiming to verify the importance of them.

Material and Methods
Organs from 29 fetuses, previously tested for PCV2, from 11 swine farms, were processed to detect PCV1, TTV1 and TTV2 nucleic acids. Samples of heart, lung, liver, kidney, lymphoid organs and nervous tissues were tested by nested-PCR. Nested-PCR reactions were performed using specific primers to detect sequences of PCV1, TTV1 and TTV2 as previously described (3, 4).

Results
Organs samples of 29 fetuses were tested by nested-PCR and PCV1 DNA was detected in 8 (27.6%) of them. In addition, TTV1 and TTV2 was detected in 7 (24.1%) and 24 (82.8%) fetuses respectively (Figure 1), and viral sequences were found in several tested organs.

Discussion
These results show the occurrence of TTV1 and TTV2 in analyzed samples, indicating its importance of TTV co-infections with PCV2. In swine, TTV alone has not showed to be pathogenic, but, its role in co-infections with other pathogens has been investigated.

Recent studies showed the first indications of TTV in utero transmission to piglets (6). Other studies claim that TTV is widely spread in Spain and could be associated with PCV2 infections (5). Porcine TTV infections are also widespread in herds of the USA, Canada, China, Korea and Thailand (9). Multiple infections of novel four U.S prototypes of porcine TTV in a single pig also have reported (10), suggesting that these co-infections are important for disease severity. The co-infection of TTV and PCV2 in piglets from Brazilian herds has also been reported (2). These findings raise the question of the importance of PCV and TTV co-infection in the pathology of PCVAD associated reproductive failures and vertical transmission in swine farms.

Figure 1. Detection of PCV1, TTV1 and TTV2 DNA in organs samples from PCV2 positive fetuses.

References
Relationship of histopathology and PCV2 detection in fetal and neonatal myocardium from cases of reproductive failure in sows

Karina Enriquez; Victor Quintero; Ignacio C. Rangel-Rodriguez; Yolanda Romero; Miguel A. Perez-Razo; Lucia A. Garcia-Camacho
FESC-UNAM, Cuautitlán, MEX, Mexico

Introduction
Porcine Circovirus type 2-associated reproductive failure (PCV2-RF) is characterized by increased abortions, stillbirths and neonatal mortality. Transplacental transmission is acknowledged and the most consistent lesion in the fetus of PCV2-RF is a non-suppurative myocarditis with intralesional PCV2 5, 6, 7 PCV2-RF has been described without co-infection with porcine parvovirus (PPV), encephalomyocarditis virus (EMCV) and porcine respiratory and reproductive syndrome virus (PRRSV)1,5 The aim of the present work was to determine PCV2 role in RF development.

Materials and Methods
In order to assess the role of PCV2 in reproductive failure in Mexico, 107 hearts (predominantly fetal samples) with presumptive diagnosis of PCV-RF were evaluated by standard procedures of histopathology and in-situ hybridization (ISH) using a digoxigenin-labelled PCR-derived probe specific for PCV2. Cases were grouped as follows: Fetuses with myocarditis (Group 1), fetuses without myocarditis (Group 2), stillborns/neonates with myocarditis (Group 3), and stillborns/neonates without myocarditis (Group 4). Among these 107 cases, 50 were selected to perform nested PCR from paraffin-embedded samples. The data were analyzed by Chi-square test, Cramer coefficient and analysis of variance for categorical data. The later was used to evaluate cardiac lesion degree and PCV2 distribution.

Results
Microscopically, 48 cases (45%) exhibited non-suppurative myocarditis, of which 35/48 were PCV2 positive (73% of cases with myocarditis, 32.7% overall) and 13 were PCV2 negative (27% of cases with myocarditis, 12.4% overall). There were 5 (4.67%) fetal cases PCV2 positive by ISH with no myocarditis. A diagnosis of myocarditis and a positive ISH showed a high relationship (J2<0.005 y Cramer coefficient= 0.64) revealing that both tests are comparable for disease diagnosis. Twenty out of 107 additional cases, regardless of myocarditis, were detected by PCR, increasing to 55 (51.4%) the cases positive to PCV2, 42/107 (38.75%) with miocarditis and 13 cases (9 fetal) without myocarditis. In total, 50 fetal cases were PCV2 positive. The myocarditis severity was mild to moderate in groups 3 y 4, and moderate to severe in fetal tissues. The lesion severity was statistically significant, showing values of P=0-04 y P= 0-0001 for 1-3 and 2-4 groups, respectively. The HIS signals were mild, variable sized and randomly distributed (Figures 4-5), precluding a statistical analysis.

Discussion
The use of histopathology as screening test is highly recommended but association to PCV2-RF must be confirmed by ISH and/or PCR. Few viral agents produce both non-suppurative myocarditis and RF1,7 Therefore, 33-39% cases are compatible to PCV2-RF, based on criteria diagnosis, and the vertical transmission was of 46.72% (50/107 fetal samples) which is higher than previous reports2,3,4,9 Cases with myocarditis but PCV2-negative suggest a separate, unknown etiology. Furthermore, no other viruses could be examined since only cardiac tissues were submitted, Therefore, co-infection cannot be entirely excluded. PCV2 relies on cell mitotic activity and DNA polymerase availability7,8,10 Our findings are in agreement, because the lesion severity was age-dependent. The ISH signals suggest low viral load. Thus, real-time PCR is being performed to assess the relationship between lesion severity and viral load.

References
Natural infection of porcine circovirus type 2 (PCV2) in fetuses from sows with reproductive failures

Giseli A. Ritterbusch2 Camila Sa Rocha3 Nelson Mores2 Neide L. Simon2 Janice R. Ciacci-Zanella1
1. Virus and Prion Diseases Research Unit, NADC, ARS, USDA, Ames, IA, USA; 2. Embrapa Swine and Poultry Research Center, Animal Healthy Laboratory, Concordia, SC, Brazil; 3. Agroveterinarian Science Center - CAV – University of Santa Catarina State – UDESC, Lages, SC, Brazil

Introduction
Porcine Circovirus Type 2 (PCV2), etiologic agent of PCVAD or PCV2 associated diseases, can cause reproductive failure in swine (1, 4, 5). PCV2 has been detected in farms with high occurrence of aborted, mummified and stillborn fetuses, and viral DNA and antigen has been detected by nested-PCR and immunohistochemistry (IHC), respectively, in organs from fetuses naturally infected (4,6). In order to study the pathogenesis of the disease in Brazilian swine herds, the objective of this work is to study the natural infection of PCV2 in organs of fetuses from sows with reproductive failures.

Material and Methods
Samples from field cases, as aborted fetuses, mummified, stillborn and fragile piglets were collected and processed in order to detect PCV2 DNA and antigens. Samples were collected from 21 farms previously identified with occurrence of reproductive losses, and a total of 169 fetuses were necropsied. Fragments of organs, including heart, lungs, liver, kidney, lymphoid organs and nervous tissues were processed for viral and histopathological diagnostic. For viral isolation, DNA was extracted and nested-PCR reactions were performed using specific primers to detect sequences of PCV2 as previously described (3). For histopathological examination, organs were fixed in 10% buffered formalin and embedded in paraffin, sectioned at 5μm thick and stained with hematoxylin and eosin for light microscopical examination. Moreover, viral presence was confirmed by IHC, carried out as previously described (2) and performed in tissues that resulted positive by PCR.

Results
Organs samples of 169 fetuses were tested by nested-PCR and PCV2 DNA was detected in 29 (17.1%) of them. Viral sequences were found in several tested organs. Microscopic lesions were found in 15.4% of the samples, mainly on heart and lymphoid organs, with the presence of infiltration of inflammatory cells, syncytial cells and varying degrees of lymphocellular depletion. In addition, IHC was performed and viral antigen was detected in 17 out of 29 positive fetuses (58.6%).

Discussion
These results show the occurrence of natural infection of PCV2 in analyzed samples, confirming the importance of PCV2 infection in reproductive failures. Among the analyzed samples, heart was the organ where PCV2 DNA was most frequently detected, following by lymphoid organs and nervous tissues, which agree with results reported by others studies (5,6). Genomic sequencing analyses are underway to classify the different PCV2 isolates. By IHC staining it was observed a variable amount and distribution of viral antigen in tissues, and positive cells were found most commonly on macrophages (Figure 1). These findings raise the question of the importance of PCVAD associated reproductive failures and vertical transmission in swine farms.

Figure 1. Detection of PCV2 by nested-PCR and IHC in organs from fetuses

References
Distribution of porcine parvovirus type 2 (PPV2, Myanmar erythrovirosis) and porcine hokovirus (PHoV) in pigs from post-weaning multisystemic wasting syndrome (PMWS)-affected and non-affected farms in archival samples from UK and Republic of Ireland

John McKillen1 John P. McKillen1 Michael McMenamy2 Irene McNair1 Gordon Allan1
1. Agri-Food & Biosciences Institute, Belfast, UK; 2. Department of Veterinary Science, The Queen’s University of Belfast, Belfast, UK

Introduction

In 2001 PPV2, a novel parvovirus related to the genus erythrovirosis, family Paroviridae was detected in swine from Myanmar. PHoV is also a member of the family Paroviridae and along with bovine hokovirus (BHov) and PARV4 form a distinct cluster within the family Paroviridae. This has been proposed as a separate genus, Hokovirus. Porcine circovirus type 2 (PCV2) is the causative agent PMWS. PMWS does not however, always occur in PCV2 infected pigs. It is likely that other agents may contribute to the development of PMWS. Here we investigate the distribution of PPV2 and PHoV in archival PMWS-affected and non-affected tissue and serum samples recovered from pigs in the UK and Republic of Ireland from 2000 to 2006.

Materials & Methods

44 sera samples were collected from pigs on PMWS-affected UK farms. Also tissues were taken from 64 PMWS-affected pigs and 55 non PMWS-affected pigs, from the UK and Republic of Ireland. Tissues included lung, liver, kidney, spleen and lymph nodes. Viral nucleic acids were extracted from sera and 10% homogenised tissue samples using Roche MagNA Pure LC. All tissue and sera samples were analysed by real-time QuantiTect SYBR Green I PCR for the presence of PPV2 and PHoV using in-house specific primers designed against deposited GenBank sequences. Between 1 and 4 tissues were tested for each animal. A selection of positive amplicons was cloned into TOPO TA vectors and sequenced using standard protocols. Sequences were compared to GenBank using nucleotide BLAST.

Results

With one exception all tissues tested positive in any positive animal. Generally PPV2 was present in significantly higher amounts than PHoV as judged by Ct values, as well as being more prevalent. Both PPV2 and PHoV were more prevalent in tissues of non-PMWS-affected pigs. Sequencing of the amplicons confirmed the specificity of the PCR. No data was available for sera from PMWS -ve farms. Tables 1 and 2 show the % of animals testing +ve for the two viruses in relation to their PMWS status.

Table 1. % of PMWS-affected and non-affected pigs with tissues testing PCR +ve for PPV2 and PHoV.

<table>
<thead>
<tr>
<th>Tissue Results</th>
<th>PPV2 +ve animals</th>
<th>PHoV +ve animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMWS +ve</td>
<td>26.6% (17/64)</td>
<td>4.9% (3/64)</td>
</tr>
<tr>
<td>PMWS -ve</td>
<td>54.5% (30/55)</td>
<td>9.1% (5/55)</td>
</tr>
<tr>
<td>Total</td>
<td>39.5% (47/119)</td>
<td>6.7% (8/119)</td>
</tr>
</tbody>
</table>

Table 2. % distribution of PPV2 & PHoV in sera from PMWS-affected farms.

<table>
<thead>
<tr>
<th>Sera Results</th>
<th>PPV2 +ve</th>
<th>PHoV +ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMWS +ve farms</td>
<td>18.2% (8/44)</td>
<td>15.9% (7/44)</td>
</tr>
</tbody>
</table>

Discussion

PHoV was detected in only 6.7% of tissue samples and 15.9% of sera samples tested in this study. In Hong Kong 44.4% of porcine samples tested +ve for PHoV. PPV2 was detected in 39.5% of the tissues tested and 18.2% of the sera, as opposed to 10.5% of tested sera from Myanmar. These preliminary studies suggest that the distribution of these viruses could be widespread and that the extent and geographical range should be further investigated. The significance of these viruses in terms of swine health is not known. Their role as potential pathogens or as co-factors in other disease complexes such as PMWS merits further investigation although higher prevalence in PMWS-affected animals and farms has not been shown in this study.

References

Reproductive failure associated to PCV2 and its distribution in the Mexican Republic

Victor Quintero; Yolanda Romero; Karina Enriquez; Lucia A. Garcia-Camacho
FESC-UNAM, Cuautitlán, MEX, Mexico

Introduction
Porcine circovirus type 2 (PCV2) has been associated to reproductive failure (RF) promoting increases of infertility abortions, stillborn, mummifications as well as a marked increase on pre-weaning mortality. The most consistent microscopic lesions are non suppurative miocarditis with perivascular and interstitial mononuclear infiltrates, degeneration and necrosis of myocytes and myocardium fibrosis. The association to PCV2-RF is confirmed by reproductive failure to PCV2 is by immunohistochemistry, in situ hybridization (ISH), and PCR in cardiac samples.1,3,5

Material and methods
The aim of this work was to determine the non-suppurative myocarditis positivity and PCV2 status as well as the locality of origin of cases presented for histopathology diagnosis in the Pathology Department of FES-Cuautitlan, UNAM during the period of 2006-2008. The heart samples were formalin-fixed and paraffin-embedded for routine microscopic evaluation. The PCV2 detection from non-suppurative myocarditis positive cases was done by ISH using a specific digoxigenin-labelled PCR-derived probe.

Results
A total of 269 heart samples from fetuses and stillborn from 39 cases from different Mexican states were microscopically evaluated. The case distribution regarding origin, myocarditis positivity and PCV2 status is shown in Table 1. Microscopically, 120 cases (44.6%) exhibited varying degree of non-suppurative myocarditis, of which 100/120 were PCV2 positive (83.3%) by ISH. The lesions consisted of perivascular and/or interstitial accumulation of lymphocytes and macrophages, myocardial degeneration, necrosis, and/or fibrosis. Additionally, subepicardial and subendocardial congestion, edema and hemorrhage were observed. Concerning the sample origin, the states with higher case frequency, such as Veracruz and Jalisco, exhibited lower proportion of myocarditis.

Discussion
Our findings strongly support the role of PCV2 as a cause of RF in Mexican farms since PCV2 was detected throughout the country. In fact, PCV2 was present in the Mexican central states, such Guanajuato, Michoacan and Jalisco where are located the main porcine production areas. Furthermore, the proportion of PCV2 in fetal and neonatal tissues is in agreement of reports elsewhere.2,5 In this study, no other viruses could be examined since the submitted samples were exclusively hearts. Therefore, co-infection cannot be entirely excluded. In fact, the low ratio of myocarditis and/or PCV2 positive cases in states with high case frequency suggests that the cause of abortion must have been different from PCV2. Thus, co-infection with other agents such as PRRSV, porcine parvovirus, Aujezsky virus and porcine rubulavirus must be considered.

Table 1. Relation of Study Cases (Period 2006-2008)

<table>
<thead>
<tr>
<th>STATE</th>
<th>N° SAMPLES</th>
<th>MYOCARDITIS (+)</th>
<th>ISH (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHIAPAS</td>
<td>18</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>EDO MEX</td>
<td>18</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>GUANAJUATO</td>
<td>17</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>HIDALGO</td>
<td>10</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>JALISCO</td>
<td>48</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>MICHOACAN</td>
<td>38</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>NAYARIT</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>PUEBLA</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>QUERETARO</td>
<td>8</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>SONORA</td>
<td>21</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>VERACRUZ</td>
<td>83</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>YUCATAN</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>TOTAL</td>
<td>269</td>
<td>120</td>
<td>100</td>
</tr>
</tbody>
</table>

References
Detection of porcine single-stranded DNA viruses in reproductive organs of culled sows

Camila Sa Rocha2, 3 Giseli A. Ritterbusch2, 3 Nelson Mores3 Neide L. Simon3 Leonardo Diniz-Mendes4 Christian Niel4 Janice R. Ciacci-Zanella1

1. Virus and Prion Diseases Research Unit, NADC, ARS, USDA, Ames, IA, USA; 2. Agroveterinarian Science Center – CAV – University of Santa Catarina State – UDESC, Lages, SC, Brazil; 3. Embrapa Swine and Poultry Research Center, Animal Healthy Laboratory, Concordia, SC, Brazil; 4. Oswaldo Cruz Institute, Rio de Janeiro, Brazil

Introduction

Several reasons determine the removal of sows from a swine herd. Sows are culled when they are considered unsuitable for further production and reproductive failure is the predominant cause, ranging between 13 – 49% of removals (1). Infectious causes of reproductive failures vary, however many can be due to viral agents (1). Among them the single stranded DNA (ss-DNA) viruses play an important role in the co-infections of these diseases. Porcine circovirus 2 or PCV2 (2), porcine parvovirus or PPV (7) and porcine torque teno virus (TTV) with at least two genogroups, TTV1 and TTV2 (3) have been identified in swine fetuses and sows tissues as the cause of infectious reproductive failures. Although a member of the Circoviridae family, PCV1 has not been associated with pathologies in pigs, but it is also an agent to be included in co-infections. Other viral agents, such as porcine reproductive and respiratory virus (PRRSV) can cause significant losses, but are not present in Brazilian swine herds (4). Thus, the objectives of this work is to detect the infection by porcine ssDNA viruses, PCV2, PCV1, PPV, TTV1 and TTV2 in reproductive organs of culled sows, aiming to verify the importance of them in those sites.

Material and Methods

Reproductive organs from 83 culled sows, from four slaughterhouses of Santa Catarina State, were collected. The reason of their removal was not particularly due to reproductive failures. A total of 71 samples of ovarian follicular fluid (OF) and 83 fragments of ovaries and 83 fragments of uterus were processed for DNA extraction followed by amplification of PCV2, PCV1, PPV, TTV1 and TTV2 nucleic acids. Nested-PCR reactions were performed using specific primers as previously described (5, 6, 7).

Results

Organs samples such as ovaries, uterus and ovarian follicular fluids (OF) of 83 culled sows were tested individually by nested-PCR for ssDNA viral amplification of PCV2, PCV1, PPV and TTVs. PCV1 DNA was detected in 14 (16.9%) of sows organs. Nucleic acids of PCV2 were amplified from 5 (6.0%) sows. TTV1 and TTV2 were detected in 25 (30.1%) and 41 (49.3%) sows respectively. For TTV2 particularly, 27 samples (38%) tested positive in OF, 19 (23%) in ovaries and 14 (17%) in uterus. Viral sequences of PPV were amplified in 6 (7.2%) sows. In some cases, the occurrence of co-infections between viral agents in sampled sows was observed.

Discussion

The present study demonstrates the occurrence of infection of porcine ssDNA viruses in reproductive organs of culled sows. These results show higher prevalence of TTV1 and TTV2 in analyzed samples, indicating its importance, mainly in co-infections with PCV2. TTV2 is more frequently related to PCV2 associated diseases (PCVAD) compared to TTV1 in Spain (77% prevalence) (5). Other reports indicate that TTV1 viral infection facilitates PCVAD (8). In swine, TTV alone has not showed to be pathogenic, but, its role in co-infections with other pathogens has been investigated. Recent studies showed the first indications of TTV in utero transmission to piglets (3). PPV has been associated with PCV2 and other swine virus such as PRRSV in samples of piglets born from sows with reproductive failure from China and Korea (9, 10). These findings raise the question of the importance of these viral infections in the pathology of reproductive failures and vertical transmission in swine.

References

Diagnostic of postweaning multisystemic wasting syndrome in pigs in Cuba

Patricia Dominguez Perez1 Humberto Ramirez-Mendoza1 Carlos Bulnes Goicochea2 Lester Josue Perez 1 Maria Iriam Percedo2 Daine Chong2 Jose Francisco Rivera-Benitez1
1. Departamento de Microbiologia E Immunologia, FMVZ-UNAM, Distrito Federal, DF, Mexico;
2. Centro Nacional de Sanidad Agropecuaria, La Habana, Cuba

Introduction
Porcine circovirus diseases (PCVDs) are nowadays considered as a significant international problem due to increased mortality rates and reduced feed conversion efficiency in weaning and fattening pigs at the worldwide (Segalés et al., 2005). The Post-weaning multisystemic wasting syndrome PMWS is economically the most important disease included within this group. This new disease syndrome was firstly identified in Canada in 1991 (Clark, 1997; Harding, 1997) since then, the PWMS has been reported in many part of the word (LeCann et al., 1997; Segalés et al., 1997; Kennedy et al., 1998).

Materials and Methods
Thirty three pigs from five farms were investigated. Clinical signs evaluated included body condition, presence of respiratory and digestive clinical signs (coughing, dyspnea and diarrhoea), behaviour (depression), skin pallor, and enlargement of superficial inguinal lymph nodes. The farms were located in three different provinces of Cuba. Affected pigs were aged 7–15 weeks. Morbidity on the five farms ranged from 10–25%; mortality was at least 50% of affected animals on all farms. Samples of tonsil, lung, kidney, spleen, jejunum, ileum, colon, liver and lymph nodes (including superficial inguinal and mesenteric) were collected and fixed by immersion in 10% neutral buffered formalin. One section was processed for routine histopathology and stained with haematoxylin and eosin. The second tissue section was processed for PCV2 nucleic acid detection by in situ hybridisation (ISH). On the other hand the other section was processed for immunohistochemistry. Tissues were evaluated for presence or absence of the PMWS (Segalés et al., 2005) specific lesions.

Results
The discoveries anatomophatology included the widespread increase of the lymph nodes, especially superficial inguinal and mesenteric, witnesses of whitish diffuse stains in the liver and kidneys and, consolidation of the lobes lung. Histopathological changes were characterised by depletion of lymphocytes in the interfollicular areas and follicular centres. Infiltration by macrophage and multinucleate giant cells were also observed in the lymph nodes.

Porcine circovirus (PCV-2) antigen and nucleic acid were regularly found in lymphoid organs. In lymph nodes, depletion was observed within lymphoid follicles or in the paracortical zones. PCV-2 antigen was detected in 23 pigs. In all tissues examined was most frequently seen in the cytoplasm of cells. Nuclear labelling was less commonly seen but occasionally appeared to be almost as abundant as cytoplasmic labelling. The smooth muscle trabeculae of the spleen were occasionally labelled. PCV-2 nucleic acid was detected in most of the pigs was found mostly in the cytoplasm of infected cells, and to a lesser extent in the nucleus.

Discussion
This is the first diagnostic of the PMWS in Cuba, based on all three accepted diagnostic criteria (Segalés et al., 2005) for the case definition of this disease syndrome. The pigs evaluated showed the clinical signs compatible with this illness. Besides, lesions on lymphoid tissues were found. Moreover, the presence of the PCV2 within these lesions in moderate or high amounts of the virus was observed. This study describes the pathological findings in 23 natural cases of PMWS, together with the tissue distribution of PCV antigen and nucleic acid. Pigs were classified as cases of PMWS on the basis of macroscopical and microscopical lesions, and the detection of PCV antigen or nucleic acid (Segalés et al., 1997).

In Cuba, PCV2 infections of the swine herds have been described recently (Pérez et al., 2009). In addition, co-infection PCV2/PPV in several farms of the different geographic region of the country was found (Pérez et al., 2009). On the other hand, classical swine fever is an endemic disease in Cuba. The use of vaccination is an important tool for combat against outbreaks of this disease. Nevertheless, if the vaccinations against classical swine fever virus can act as trigger fact of PMWS will be needed to be explained.

References
First evidence of porcine circovirus type 2 in pigs from extensive system raising from Goiás State, Brazil

Denise L. Barthasson1 Patricia Soares2 Keili M. Souza3 Daniel Linhares1 Paulo M. Roche4 Jurij Sobestiansky2 Wilia M. Brito3

1. College of Veterinary Medicine, University of Minnesota, Saint Paul, MN, USA; 2. Programa de Pós graucação em Ciência Animal, Escola de Veterinária da Universidade Federal de Goiás/GO, Goiania, GO, Brazil; 3. Laboratório de Virologia Animal, Instituto de Medicina Tropical e Saúde Pública da Universidade Federal de Goiás, Goiania, GO, Brazil; 4. Setor de Virologia, Instituto de Pesquisas Veterinárias Desidério Finamor, RS, Porto Alegre, RS, Brazil

Introduction

Porcine circovirus type 2 (PCV2) is ubiquitous in swine herds over the world. It is associated with different disease and syndromes in pigs mainly to postweaning multisystemic wasting syndrome (PMWS). In Brazil, PCV2 was first identified in South region, in 2000 and in other regions later on. All Brazilian studies were conducted in commercial and intensive raising systems animals (Ciacci-Zanella et al., 2003; Ruiz et al., 2004). In peripheral areas of large cities in Brazil, it is common to create a few pig herds (backyard pigs), for own consumption, under poor hygienic and sanitary conditions and in close contact with others animals species and humans. This swine population is a unique type of extensive raising swine system (ERSS). At our knowledge, no other study was done to verify PCV2 infection in these swine population. The present study was performed to identify PCV2 DNA in clinical specimens of pigs in Goiás State, Brazil ERSS’s.

Material and Methods

A total of 200 serum samples from swine of different age groups were collected from 25 ERSS in Goiás state. Tissue samples (lymph nodes, lungs, liver, brain, kidney and spleen) from 5 pigs with growth impairment and 6 pigs with signs of neurological disease were also included. Viral DNA was extracted with standard phenol-chloroform-isooamyl alcohol extraction procedure. Previous described specific primers to ORF2 region of PCV2 (F5′-CGGATATGGTAGTCTTGTTGCG-3′) and (R5′-ACTGTCAAGGCTACCCAGTC A-3′) and PCR conditions were used for DNA PCV2 identification (Kim et al., 2001). A fragment of 480pb length was expected.

Results and Discussion

Out of the serum samples, 57 (28.5%) were positive. This frequency is lower than that observed in other studies (Casalmiglia et al., 2002; Grau-Roma et al., 2008) although it may represent the occurrence of PCV2 infection in this particular type of swine herd. Seven (63.6%) of the eleven animals tested showed positivity in at least one tissue sample. Viral DNA was found in inguinal nodes (6/7), lung (1/7) and kidney (1/7). Other authors also found high frequency of identification of PCV2 DNA in inguinal lymph nodes and assert that they are one the main targets for PCV2 replication (Yu et al., 2007). Despite viral DNA had been found in two fragments of pigs with growth impairment it is not enough to ensure the occurrence the syndrome. This also should be associated with clinical signs and pathological findings (Segalés et al., 2003). The results here presented may point out that PCV2 occur in pigs under extensive raising system in Goiás, Brazil.

References


Detection of TTV2 and PCV2 in a high health swine herd located São Paulo State, Brazil

Alessandra M. Castro1 Cíntia M. Favero1 Fernando G. de Castro2 Tais Nishikata1
Cíntia M. Baldin3 Paulo E. Brandão1 Leonardo J. Richtzenhain1
1. Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, São Paulo, Brazil; 2. Instituto de Zootecnia, Nova Odessa, Brazil; 3. Faculdade de Jaguariúna, Jaguariúna, SP, Brazil

Introduction
Torque Teno virus (TTV) is a non-enveloped, circular, single stranded DNA virus that may infect human, non-primate and domestic species including swine. Two TTV genogroups (TTV1 and TTV2) have been described in pigs. TTV is considered non-pathogenic, but a recent study has shown an increased prevalence of TTV2 in PCV2-associated diseases compared to non-affected ones (2,3). In Brazil PCV2-associated disease is expected to diminish with the introduction of PCV2 vaccination. However, PCV2-associated disease is still a problem in swine herds. Since the main virus, PRRS, involved in the co-infection of PCV2 to the development of the disease, is absent in Brazil, other agents became important. Nowadays, there are few information about TTV in Brazilian swine herd. Therefore, the aim of this work is to describe, for first time, the detection of co-infections of TTV2 and PCV2 in a high health swine herd.

Materials and Methods
Twenty and six serum samples were collected from sows from a high health herd located in São Paulo state, Brazil. DNA was extracted from serum using the phenol:chloroform Proteinase K protocols. TTV DNA was amplified a fragment of 250 bp using a PCR with primers targeting two conserved domains within an untranslated region of the viral genome (4). The detection of PCV-2 was performed by PCR using primers Fa/Ra that amplify a 476 bp fragment (1). One sample was randomly selected and sequenced. Nucleotide sequences were aligned with sequences contained at the GenBank database of the swine TTV and phylogenetic tree constructed.

Results
TTV2 was detected in 14 (58.3 %) out of the 24 serum samples examined. Among the 14 TTV2 positive samples, five (5/9) was co-infected with PCV2. PCV2 alone was detected in five sow serum samples (Fig. 1). The alignment of the TTV2 sequence obtained in this study showed nucleotide and amino acid identities values of 91.6 % to 82.4 % and 61.0 to 64.6 % with available GenBank sequences of TTV2 and TTV1, respectively. A Neighbour-Joining tree was assembled and showed that this sequence had been classified TTV2 group (Fig. 2).

Discussion
This is the first report of TTV-2 and PCV2 co-infections in Brazilians pigs. The alignment of one sample confirms that the TTV detected belongs to group 2. These results, also, demonstrated that TTV2 is circulating in high health swine herds in Brazil. Previous research showed higher prevalence of TTV2 in PMWS-affected pigs than in healthy ones (2). The involvement of TTV2 in the development of diseases associated with PCV2 is not clear, but the absence of PRRS in Brazil and the low detection of PPV in PCV2-associated disease, make TTV a possible candidate to be involved in disease cases.

References