Porcine circovirus type 2 in muscle and bone marrow is infectious and transmissible to naïve pigs by oral consumption

Tanja Opriessnig a,*, Abby R. Patterson a, Xiang-Jin Meng b, Patrick G. Halbur a

a Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, 1600 South 16th Street, Ames, IA 50011, USA
b Center for Molecular Medicine and Infectious Diseases, College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

Received 16 March 2008; received in revised form 13 June 2008; accepted 26 June 2008

Abstract

Pork products are a possible source of introduction of PCV2 isolates into a pig population. However, limited work has been done on the transmission through meat of porcine circovirus type 2 (PCV2), a virus associated with several disease syndromes in pigs. The objectives of this study were to determine if pork products from PCV2-infected pigs contain PCV2 DNA/antigen and to determine if the PCV2 present in the tissues is infectious by performing in vitro and in vivo studies. Skeletal muscle, bone marrow, and lymphoid tissues from pigs experimentally inoculated with PCV2 were collected 14 days post-inoculation (DPI). The tissues were tested for presence of PCV2 DNA by quantitative real-time PCR, for PCV2 antigen by immunohistochemistry (IHC), and for presence of infectious PCV2 by virus isolation and inoculation of PCV2 naïve pigs. Lymphoid tissues contained the highest amount of PCV2 (positive by PCR, IHC, and virus isolation), bone marrow contained a lower amount of PCV2 (positive by PCR and IHC but negative by virus isolation), and skeletal muscle contained the lowest amount of PCV2 (positive by PCR but negative by IHC and virus isolation). Naïve pigs fed for three consecutive days with either skeletal muscle, bone marrow, or lymphoid tissues all became PCV2 viremic as determined by quantitative real-time PCR on serum starting at 7 DPI. The pigs also seroconverted to PCV2 as determined by PCV2 IgM and IgG ELISA. In addition, PCV2 antigen was detected by IHC stains in lymphoid tissues and intestines collected from the majority of these pigs. Results from this study indicate that uncooked PCV2 DNA positive lymphoid tissues, bone marrow, and skeletal muscle from PCV2 viremic pigs contain sufficient amount of infectious PCV2 to infect naïve pigs by the oral route.

© 2008 Elsevier B.V. All rights reserved.

Keywords: Porcine circovirus type 2; Transmission; Meat; Bone marrow; Pigs

* Corresponding author. Tel.: +1 515 294 1137; fax: +1 515 294 3564.
E-mail address: tanjaopr@iastate.edu (T. Opriessnig).
1. Introduction

In 2006, the United States exported 1,262,499 metric tons of pork products to various countries including Japan, Mexico, South Korea, Canada, Hong Kong/China, Taiwan and other countries (http://www.usmef.org/TradeLibrary.pdf; U.S. Meat Export Federation: Total U.S. Exports 1996–2006; accessed 26 February 2008). Comparatively, only 422,373 metric tons of pork products originating in the United States were exported in 1996. While the increase in exportation of pork products is beneficial to the United States swine industry, it also increases the potential for market disruption should restrictions be placed on imports from the United States due to the real or perceived risk of pork products transmitting diseases between countries. As such, the potential hazard of transmitting emerging porcine pathogens into naïve pig populations through the importation of pork products requires evaluation.

In a 1997 publication, information on agent survivability in pork and pork products was reported for several porcine pathogens including African swine fever virus, classical swine fever virus, foot and mouth disease virus, porcine reproductive and respiratory syndrome virus (PRRSV), swine vesicular disease virus, and transmissible gastroenteritis virus (Farez and Morley, 1997). Studies have also been published on the presence of PRRSV in packaged meat (Larochelle and Magar, 1997), in non-processed pig meat (Cano et al., 2007), and on the ability of PRRSV to be orally transmitted to pigs through infected muscle tissues (van der Linden et al., 2000; Magar and Larochelle, 2004). No information is currently available on porcine circovirus type 2 (PCV2), a small, non-enveloped, single stranded DNA virus (Tischer et al., 1982) which emerged in the 1990s as a swine pathogen (Allan et al., 1998). Besides PCV2, the family Circoviridae also contains porcine circovirus type 1 (PCV1) which has been experimentally shown to be non-pathogenic to pigs (Tischer et al., 1986). In contrast, PCV2 is associated with several disease complexes in pigs commonly referred to as porcine circovirus type 2 associated disease (PCVAD) characterized by hallmark lymphoid lesions (Sorden, 2000). Recently it has been shown that there are several PCV2 genotypes (Grau-Roma et al., 2008) and there are concerns that a non-resident PCV2 strain could be introduced into an area by means of pork products.

PCV2 is commonly demonstrated in lymphoid tissues and it has also been demonstrated in bone marrow of 7 of 14 pigs from 20 to 28 days post-inoculation (DPI) and in 1 of 5 pigs at 35 DPI (Bolin et al., 2001). PCV2 has also been found in the heart tissues of growing pigs (Kennedy et al., 2000; Bolin et al., 2001). It has been determined that PCV1 is stable at a pH of 3 (Allan et al., 1994). Since meat products typically do not reach a pH lower than 6.2 there is a high likelihood that meat from PCV2-infected pigs at the time of slaughter would contain infectious PCV2 and that the PCV2 would survive the meat processing and storage. Although there is no publication available on the survivability of PCV2 in pork products, viral antigen from chicken anemia virus, another member of the Circoviridae family has been detected in chicken muscle tissues at 11 and 12 DPI and in bone marrow for up to 13 DPI (Smyth et al., 1993).

Previous studies have indicated that PCV2 transmission typically occurs by the fecal–oral route (Bolin et al., 2001; Shibata et al., 2003; Caprioli et al., 2006). Under experimental conditions, pigs are typically inoculated intranasally and/or intramuscularly; however, there are reports of successful experimental PCV2 infection via the oronasal route (Krakowka et al., 2001; Allan et al., 2000). However, questions remain as to the significance of the oral route of infection for PCV2 transmission under field conditions.

The objectives of this study were to determine if selected tissues (skeletal muscle, bone marrow, lymphoid tissues) from pigs experimentally inoculated with PCV2 contain PCV2 DNA/antigen and if the PCV2 present in the tissues is infectious by performing in vitro and in vivo studies.

2. Materials and methods

2.1. Origin of the tissues used in the study

The tissues were collected from three pigs from a previous PCV2 study (Opriessnig et al., 2006b). In brief, 7-week-old pigs were inoculated intramuscularly...
(1 ml) and intranasally (1 ml) with PCV2 isolate 40895 (approximately 10^4.7 50% tissue culture infectious dose [TCID_{50}] per pig) and were euthanized at 14 DPI. All tissues were collected at necropsy as follows: the personnel collecting the tissues changed gloves between pigs and between different tissues from the same pig. For tissue removal, sterile surgical blades (separate blades for each tissue) and scissors and forceps that were cleaned and disinfected with alcohol and flamed between tissues were used. Skeletal muscle (loin), half of the spleen, half of the tonsil, several lymph nodes (superficial inguinal, mediastinal, tracheobronchial, and mesenteric), and bone marrow (from the femur head) were collected in separate bags for each tissue and pig, cooled for 24 h at 4°C, and stored at −80°C until further use. Small portions of the tissues were placed in 10% neutral-buffered formalin and processed for histopathological examination.

2.2. DNA extraction and quantitative real-time PCR for PCV2

The DNA of the different tissues (for each individual pig of origin and on the tissue pool used for the in vivo experiment) was extracted with the DNeasy Blood and Tissue Kit (Qiagen, Valencia, California, USA) according to the manufacturer’s protocol. Serum samples were extracted with the QIAamp™ DNA mini kit (Qiagen). DNA-extracts were used for quantification of the copy number of PCV2 genomic DNA by quantitative real-time PCR as described previously (Opriessnig et al., 2003).

2.3. Immunohistochemistry (IHC)

IHC for detection of PCV2-specific antigen was performed on formalin-fixed and paraffin-embedded sections of selected tissues (skeleton muscle, bone marrow, and lymphoid tissues [superficial inguinal, mediastinal, tracheobronchial, and mesenteric lymph nodes, tonsil, and spleen], and Peyer’s patches) using a rabbit polyclonal antiserum (Sorden et al., 1999). PCV2-antigen scoring was done in a blinded fashion and scores ranged from 0 (no signal) to 3 (strong signal) (Opriessnig et al., 2004).

2.4. Virus isolation

A representative portion of the tissue pools (skeletal muscle, bone marrow, lymphoid tissue pool) was homogenized with 5 ml phosphate buffered saline. Samples were tested for the presence of PCV2 by virus isolation on the continuous porcine kidney cell line (PK-15) as previously described (Pogranichniy et al., 2002). Samples were considered negative for PCV2 if no sign of virus replication was detected in cells after two blind passages. Immunofluorescence microscopy using polyclonal anti-PCV2 was used to demonstrate PCV2 within cells (Pogranichniy et al., 2002).

2.5. PCV2 in vivo infectivity: animals, housing, and experimental design

Fifteen, 2-week-old, colostrum-deprived, crossbred pigs were purchased from a herd that is routinely tested for and known to be free of PRRSV and swine influenza virus (SIV). The pigs were brought to the research facility at Iowa State University. Upon arrival, the pigs were randomly assigned to five groups and rooms with three pigs in each group. Pigs within each room were kept in an individual 1.5 m × 2 m raised wire deck equipped with one nipple drinker and a self-feeder separated by a solid 1.5 m high galvanized metal partition that prevented nose-to-nose contact with the other pigs in the room. To avoid cross-contamination between rooms, separate pig equipment and personnel was used for each of the rooms. Showering in and out of rooms accompanied by change of gloves, boots, surgical mask, and coveralls was required. The experimental design was as follows: group 1 (controls; no treatment), group 2 (skeletal muscle; orally), group 3 (bone marrow; orally), group 4 (lymphoid tissues; orally), and group 5 (PCV2 inoculum; stomach tube). The experimental protocol was approved by the Iowa State University Institutional Animal Care and Use Committee.

2.6. PCV2 in vivo infectivity: serology

Blood was collected on the farm of origin at 1 day of age, after arrival of the pigs at the research facility at 2 weeks of age, at the day of inoculation at 3 weeks of age (=DPI 0), and on 7, 14, 21, and 28 DPI.
2.6.1. Anti-PCV2-IgG-antibodies

The serum samples were tested by an IgG ELISA based on PCV2 open reading frame 2 (ORF2) as previously described (Nawagitgul et al., 2002). Samples were considered positive if the calculated sample-to-positive (S/P) ratio was 0.2 or greater.

2.6.2. Anti-PCV2-IgM-antibodies

All serum samples were tested by the Ingezim PCV2 ELISA IgM assay (Ingenasa, Madrid, Spain). The ELISA cut-off value was determined by multiplying the average optical density at 450 nm of the IgM positive control wells with 0.4.

2.6.3. PRRSV, porcine parvovirus (PPV), and SIV serology

The serum samples from all pigs taken at necropsy were tested for the presence of antibodies to PRRSV by PRRSV-ELISA (IDEXX Laboratories, Inc. Westbrook, Massachusetts, USA), PPV by hemagglutination inhibition (HI) assay (Mengeling et al., 1988), and H1N1 SIV and H3N2 SIV by HI assays according to the protocol used at the Veterinary Diagnostic Laboratory at Iowa State University.

2.7. PCV2 in vivo infectivity: inoculation

Inoculation was done on three consecutive days with the same protocol each day: all pigs in all groups were deprived of food for 12 h prior to each inoculation but were allowed full access to water. The inocula (pork products and PCV2 inoculum) were moved from −80 to 4 °C 24 h prior to inoculation.

2.7.1. Pork products (groups 2–4)

Each pig received a clean cup with approximately 100 g uncooked, chopped skeletal muscle (group 2), approximately 2.2 g of uncooked, chopped bone marrow (group 3), or approximately 45 g uncooked, chopped lymphoid tissues (lymph nodes [superficial inguinal, mediastinal, tracheobronchial, and mesenteric], tonsil, and spleen; group 4) prior to feeding for three consecutive days. Each pig was monitored until the entire amount of tissue was consumed. This typically took 3–5 min. Once finished, the pigs received their regular feed. This ensured that all pigs consumed similar amounts of tissue.

2.7.2. Infectious PCV2 virus (group 5)

A homogenous infectious stock of PCV2 isolate 40895 (Fenaux et al., 2000) was generated via direct transfection of PK-15 cells with an infectious clone of PCV2 as previously described (Fenaux et al., 2002). The virus inoculum contained infectious PCV2 at a titer of $10^{5.2}$ TCID$_{50}$ per ml. The inoculum was tested negative for the presence of PPV or PCV1 nucleic acids by PCR (Fenaux et al., 2000; Kim et al., 2001). Group 5 pigs received 2 ml of the PCV2 inoculum through an oral gastric tube placed into the stomach each morning before feeding for three consecutive days.

2.8. PCV2 in vivo infectivity: clinical evaluation

Following PCV2-inoculation, the pigs were monitored daily and scored for severity of clinical respiratory disease ranging from 0 (normal) to 6 (severe dyspnea and abdominal breathing) (Halbur et al., 1995).

2.9. PCV2 in vivo infectivity: necropsy

Necropsy was conducted on all pigs on 28 DPI. The total amount of macroscopic lung lesions ranging from 0 to 100% of the lung affected and size of lymph nodes ranging from 0 (normal) to 3 (four times the normal size) were estimated in a blinded fashion (Opriessnig et al., 2004). Lungs were insufflated with fixative. Sections of lymph nodes (superficial inguinal, mediastinal, tracheobronchial, mesenteric), tonsil, thymus, ileum, kidney, colon, spleen, and liver were collected at necropsy and fixed in 10% neutral-buffered formalin and routinely processed for histological examination.

2.10. PCV2 in vivo infectivity: microscopic evaluation

Microscopic lesions were evaluated by a veterinary pathologist blinded to the treatment status. Lung sections were scored for presence and severity of interstitial pneumonia ranging from 0 to 3 (0 = normal; 3 = severe) (Halbur et al., 1995). Sections of heart, liver, kidney, ileum, and colon were evaluated for the presence of lymphohistiocytic inflammation and scored from 0 to 3 (0 = none to 3 = severe). Lymphoid tissues including lymph nodes, tonsil, and spleen were evaluated for the presence of lymphoid
depletion ranging from 0 (normal) to 3 (severe) and histiocytic inflammation and replacement of follicles ranging from 0 (normal) to 3 (severe) (Opriessnig et al., 2004).

2.11. PCV2 in vivo infectivity: statistical analysis

Summary statistics were calculated for all groups to assess the overall quality of the data, including normality. PCR data (continuous data) were analyzed using analysis of variance (ANOVA). Histopathology data (non-parametric data) were assessed using non-parametric Kruskal-Wallis ANOVA. The significance level used was \( P < 0.05 \). Statistical analysis was performed using JMP 6.0.0 (SAS Institute, Inc., Cary, NC).

2.12. Sequencing

PCR products amplified from virus recovered from one randomly selected pig at 28 DPI from each inoculation group were sequenced and compared to the PCV2 inoculum or to the PCV2 sequence present in the skeletal muscle, bone marrow, or lymphoid tissue pool used for inoculation. Nested PCR was used to amplify the entire ORF2 gene for sequencing and sequence comparison (Opriessnig et al., 2006b). The PCR products were run on a 1% agarose gel and the expected 820 bp products were excised, purified and sequenced at the Virginia Bioinformatics Institute at Virginia Tech using an Automated DNA Sequencer (Applied Biosystems Inc., Foster City, California, USA). The sequences were analyzed with the MacVector computer program and compared to the sequences of the original virus inocula.

3. Results

3.1. Incidence and amount of PCV2 in the tissues of origin

Pooled lymphoid tissues, bone marrow, and skeletal muscle were positive for PCV2 DNA with \( 10^{7.8}, 10^{5.8}, \) and \( 10^{4.0} \) PCV2 DNA genomic copies per ml of homogenized tissues, respectively.

Immunohistochemical examination of the tissues from experimentally inoculated pigs used in this study demonstrated a large amount of PCV2 antigen in pooled lymphoid tissues (IHC score 3), low-to-moderate amounts in bone marrow (IHC scores 1–2), and absence of PCV2 antigen in skeletal muscle (IHC score 0).

3.2. PCV2 in vitro infectivity

Virus isolation attempts for PCV2 from skeletal muscle and bone marrow of Phase I pigs were negative after two blind passages. Virus isolation attempts for PCV2 from Phase I pigs pooled lymphoid tissues were positive after the first passage.

---

Fig. 1. Group mean optical density ratios and standard errors for anti-PCV2-IgM antibody response.
3.3. PCV2 in vivo infectivity: presence of anti-PCV2 IgM and IgG antibodies

The mean group anti-IgM PCV2 antibody response is summarized in Fig. 1. One of three pigs in group 3 (bone marrow) and three of three pigs in groups 4 (lymphoid tissues) and 5 (PCV2 via stomach tube) had positive OD values by 14 DPI (Fig. 1). All pigs in groups 2–5 had positive anti-PCV2 IgM OD values at 21 and 28 DPI.

The mean group anti-IgG PCV2 antibody response is summarized in Fig. 2. In groups 4 (lymphoid tissues) and 5 (PCV2 via stomach tube), two of three pigs seroconverted to PCV2 IgG antibodies by 14 DPI (Fig. 2) and all six pigs in these groups had S/P ratios above the cut-off on DPI 21 and 28. In group 2 (skeletal muscle), one of three pigs seroconverted by DPI 21 and three of three pigs in this group were seropositive on DPI 28. In group 3 (bone marrow) all three pigs remained seronegative until DPI 28 when three of three pigs seroconverted.

At 28 DPI, there were no detectable antibodies against PRRSV, H1N1 SIV, H3N2 SIV, or PPV in any of the serum samples.

3.4. PCV2 in vivo infectivity: viremia

All pigs were negative for PCV2 DNA on serum on the day of inoculation. Oral consumption of lymph node, bone marrow, and skeletal muscle resulted in
PCV2 viremia by DPI 7 (Fig. 3). Specifically, one of three pigs in group 2 (skeletal muscle), one of three pigs in group 3 (bone marrow), three of three pigs in group 4 (lymphoid tissues), and two of three pigs in group 5 (PCV2 via stomach tube) were PCR positive for PCV2 DNA on DPI 7. All 12 pigs in groups 2, 3, 4, and 5 were PCR positive for PCV2 DNA on serum through termination of the study (Fig. 3). Pigs in group 1 (negative controls) remained negative throughout the trial. The amount of PCV2 DNA in serum samples was not different \((P > 0.05)\) between groups at any of the DPIs. Based on ORF2 sequencing results, the sequence of the PCV2 present at 28 DPI was 100% identical with that of the PCV2 present in the tissues used for inoculation.

3.5. PCV2 in vivo infectivity: presence of PCV2 antigen in lesions of affected tissues

Microscopic lesions consistent with PCV2-infection characterized by mild lymphocytic depletion and histiocytic replacement of follicles were observed in two of three pigs in each of groups 2 (skeletal muscle), 3 (bone marrow), and 5 (PCV2 via stomach tube) and in three of three pigs in group 4 (lymphoid tissues). Low-to-moderate amounts of PCV2 antigen were associated with the microscopic lesions. The IHC results for individual lymphoid tissues are summarized in Table 1. There were no significant \((P > 0.05)\) differences in amount of PCV2 antigen among groups.

### Table 1

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Lymph nodes</th>
<th>Tonsil</th>
<th>Spleen</th>
<th>Peyer’s patches</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>0/3 (0.0)</td>
<td>0/3 (0.0)</td>
<td>0/3 (0.0)</td>
<td>0/3 (0.0)</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>2/3 (1.5 ± 0.5)</td>
<td>1/3 (1.0)</td>
<td>2/3 (1.5 ± 0.5)</td>
<td>1/3 (1.0)</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>1/3 (1.0)</td>
<td>1/3 (1.0)</td>
<td>1/3 (1.0)</td>
<td>1/3 (1.0)</td>
</tr>
<tr>
<td>Lymphoid tissues(^a)</td>
<td>3/3 (1.0)</td>
<td>1/3 (2.0)</td>
<td>1/3 (1.0)</td>
<td>1/3 (1.0)</td>
</tr>
<tr>
<td>PCV2</td>
<td>2/3 (2.0)</td>
<td>2/3 (1.5 ± 0.5)</td>
<td>2/3 (1.0)</td>
<td>1/3 (2.0)</td>
</tr>
</tbody>
</table>

\(^a\) Lymph nodes (superficial inguinal, mediastinal, tracheobronchial, and mesenteric), tonsil, and spleen.

Data presented as number of pigs/total number of pigs per group (mean positive score ± standard error if applicable).

4. Discussion

To our knowledge, this is the first study confirming that PCV2 can be transmitted by the gastroenteric route. Because a stomach tube was used for the PCV2 inoculum, it is unlikely that the virus might have entered through the tonsils rather than through the gastrointestinal mucosa. Furthermore, the results of this study indicate that PCV2 can be transmitted to naïve pigs by oral inoculation with PCV2 DNA positive, uncooked skeletal muscle, bone marrow, or lymphoid tissue homogenate.

Our hypothesis was that skeletal muscle from PCV2-infected pigs does not contain infectious PCV2 since skeletal muscle is not known to be a target tissue for PCV2 replication in growing pigs. The hallmark lesions of PCVAD are PCV2-associated lymphoid depletion (Sorden, 2000) and the tissues where PCV2 is usually found in highest frequency and numbers are lymphoid tissues. Therefore, lymphoid tissues were used as an additional positive control. The experimentally inoculated pigs, from which the tissue inoculum was derived, were necropsied at the anticipated peak PCV2 viremia stage. As described previously (Opriessnig et al., 2006b), none of the pigs developed clinical disease during the 2-week duration of the experiment. Development of clinical disease at a later time point cannot be ruled out.

We purposely selected tissues for inocula from pigs with high amounts of PCV2 antigen in lymphoid tissues. These lymphoid tissues from the experimentally inoculated animals contained large amounts of PCV2 and the PCV2 was readily isolated in cell culture from those tissues. Bone marrow contained lower amounts of PCV2 DNA; however, the PCV2 antigen was still detectable by IHC staining. In contrast, skeletal muscle contained the lowest amount of PCV2 DNA, and PCV2 IHC and virus isolation were both negative on skeletal muscle samples. Previously, it has been shown that an estimated virus load of \(10^9\) PCV2 genomes per 500 ng DNA was required to give visible IHC staining in tissue sections.
In addition, virus isolation for PCV2 is difficult. It is well documented that PCV2 grows slow and needs specific culture conditions (Tischer et al., 1987). Even under optimized culture conditions, PCV2 isolation is not always successful. Therefore, it is not really surprising that the in vivo study was a more sensitive test to detect the presence of viable PCV2 in tissues with low amount of PCV2 such as bone marrow and skeletal muscle.

Based on viremia levels and the onset of appearance of seroconversion, the infectivity of the PCV2 present in the lymphoid tissues was comparable to the PCV2 virus positive control inoculum. The pigs inoculated with the skeletal muscle or the bone marrow had delayed anti-IgM and anti-IgG responses suggesting that it took longer for the virus in these inocula to replicate to a level sufficient to induce a measurable serological immune response. However, the skeletal muscle clearly contained a sufficient amount of PCV2 to successfully infect naive pigs by oral consumption of the uncooked pork product. It needs to be noted that group 3 pigs (bone marrow) received only approximately 7.5 g of bone marrow over a 3-day-period due to limited availability of bone marrow, whereas each of the group 2 pigs (skeletal muscle) received and consumed approximately 300 g skeletal muscle over the inoculation period.

Sequencing was done on the virus present in the meat products and in the inoculum. These sequences were compared to the PCV2 in the inoculated pigs at the termination of the study. While this approach helps to determine if the pigs became infected by a different PCV2 (present in the rooms, feed, pig source, etc.), it cannot be used to rule out cross-contamination between rooms. It would have been possible to use a different PCV2 isolate for the PCV2 group 5; however, we wanted to make sure that the pigs used are susceptible to infection with the particular PCV2 isolate present in the meat. A rigorous biosecurity protocol between rooms was implemented and the negative control group 1 remained PCR and ELISA negative for PCV2 supporting the fact that room cross-contamination was probably not an issue in this study.

Similar studies as the one described in this study have been conducted with PRRSV and it was found that muscle from pigs experimentally inoculated with PRRSV contains detectable PRRSV RNA. It has been suggested that the low levels of PRRSV were due to residual blood rather than active infection of muscle cells (Bloemraad et al., 1994; Magar et al., 1995; van der Linden et al., 2003). The same may also be true for PCV2 since PCV2 in the acute phase of infection can be found in high amounts in serum. However, it is also known that PCV2 replicates in fetal heart muscle (Sanchez et al., 2003) and it has been suggested that PCV2 replicates in endothelial cells (Oppriessnig et al., 2006a). Therefore, it is entirely possible, that PCV2 replicates in low levels in muscle and/or associated blood vessels.

Under the conditions of the current study, it appears there is risk of transmission of PCV2 via consumption of uncooked meat. In this study, we used tissues collected from experimentally infected pigs at the expected peak PCV2 load. Although there is limited data available, it is generally believed that the majority of pigs at slaughter age would likely be well beyond the acute stages of infection. However, there is evidence in the literature that PCV2 infection can potentially result in long-term viremia (Bolin et al., 2001) and PCV2 may still be present at slaughter (Liu et al., 2002; Rodríguez-Arrioja et al., 2002). In this situation, the amount of PCV2 may be much lower than in the tissues from the pigs in the current study which were at expected PCV2 peak viremia. The minimal infectious dose for PCV2 is unknown and it is therefore currently unknown if a lower amount of PCV2 in muscle tissue would still be sufficient to cause PCV2 infection in naive pigs by using the oral route.

Future studies need to investigate the presence and amount of PCV2 in fresh and frozen packaged meat from slaughter age pigs in order to further assess the risk of pork products in PCV2 transmission. Work done with PRRSV in packaged pig meat using virus isolation and reverse transcriptase PCR methods concluded that PRRSV was undetectable in those products and the authors concluded that pig meat does not retain detectable amounts of PRRSV and transmission of PRRSV via pig meat is unlikely (Larochelle and Magar, 1997). A study investigating another RNA virus, swine hepatitis E virus (HEV), in packaged and frozen liver found that the HEV induced viremia and seroconversion when administered to naive pigs, thus was determined to be infectious (Feagins et al., 2007). PCV2 as a non-enveloped small DNA virus is expected to have a greater thermal
stability and freezing usually is not sufficient to affect PCV2 viability (Ellis et al., 1998).

In contrast, heat treatment has been shown to sufficiently inactivate a variety of viruses including PRRSV and HEV. When liver samples that contained infectious HEV were heated, the HEV was inactivated and no longer infectious (Feagins et al., 2008). The effect of cooking on viability and infectivity of PCV2 in meat and blood is currently unknown although recent research suggests that PCV2 is extremely resistant to changes in the environment. It was demonstrated that non-pathogenic PCV1 was stable at pH 3, was stable at 56 °C and at 70 °C for 15 min, and was resistant to inactivation after exposure to chloroform (Allan et al., 1994). Recently, the mean log infectivity of PCV2 was reduced by only 1.25 with a dry-heat treatment up to 120 °C for 30 min (Welch et al., 2006). However, the mean log infectivity reduction was greater than 3.2 when treated with wet-heat at 80 °C (Welch et al., 2006). Another study found that PCV2 retained infectivity when heated at 75 °C for 15 min; however, it was inactivated when heated at 80 °C for 15 min (O’Dea et al., 2008). In addition, the risk of transmission of PCV2 in blood and products derived from blood such as spray dried plasma proteins should be further assessed. Future studies need to investigate these potential means of PCV2 transmission including the effect of dose on virus infectivity and the effect of cooking or heat treatment on virus viability and risk of transmission.

Acknowledgements

The authors thank the American Pork Export Federation (APEX) for funding of this project. We also thank Dr. Pete Thomas, Matt Boogerd, and Paul Thomas for assistance with the animal work, Fort Dodge Animal Health for providing the PCV2 inoculum, and Dr. Nicole Juhan for assistance with sequencing.

References


genomic DNA of type 2 porcine circovirus is infectious when injected directly into the liver and lymph nodes of pigs: characterization of clinical disease, virus distribution, and pathologic lesions. J. Virol. 76, 541–551.


