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Short communication

Shedding and infection dynamics of porcine circovirus type 2 (PCV2) after natural exposure

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ABSTRACT

The objective of this study was to determine the amount of porcine circovirus type 2 (PCV2) shed in nasal, oral and fecal secretions over time following natural PCV2 infection. Fecal, oral and nasal swabs and blood were collected at regular intervals starting at 28 days post-farrowing (DPF) until 209 DPF from four pigs naturally infected with PCV2. PCV2 DNA was detected in all sample types. There were no differences in the amount of PCV2 DNA present in different sample types over time. PCV2 DNA was detectable in sera and secretions in pigs through 209 DPF. Natural exposure to PCV2 results in a long term infection and PCV2 is shed in similar amounts by nasal, oral and fecal routes.

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1. Introduction

Porcine circovirus type 2 (PCV2) is a small, circular, single-stranded DNA virus which was initially associated with disease in the 1990s (Harding et al., 1998). Although PCV2 has been shown to be present in the swine population worldwide, there is a lack of information on the infection dynamics of a natural PCV2 infection in herds without other viral coinfections. Previously published information has provided quantities of PCV2 DNA present in excretions and tissues from naturally infected pigs submitted to a diagnostic laboratory (Segalés et al., 2005b). The length of viremia and onset of seroconversion in naturally infected populations have also been described (Carasova et al., 2007; Grau-Roma et al., 2009). These studies demonstrated that in naturally infected populations, anti-PCV2 IgM appeared first around 8 weeks of age followed by anti-PCV2 IgG which appeared at 10 weeks of age (Carasova et al., 2007). PCV2 viral load peaked at 10 weeks of age but PCV2 viremia was present until termination of the study at 25 weeks of age (Carasova et al., 2007). In addition, it has been reported that on farms naturally affected by porcine circovirus associated disease (PCVAD) in Denmark and Spain, the PCV2 load increased as passively acquired antibody levels decreased and that the amount of PCV2 DNA present in sera correlated well with the amount present in nasal and fecal swabs in these pigs (Grau-Roma et al., 2009). However, results from these observational studies of naturally infected pigs may be influenced by pathogen and environmental co-factors that are known to increase the duration and level of PCV2 replication (Dorr et al., 2007). To our knowledge, a detailed investigation of the infection dynamics in PCV2-positive pigs without coinfection with porcine reproductive and respiratory virus (PRRSV), swine influenza virus (SIV) or parvovirus (PPV) has not been reported to date. The objective of this study was to determine and compare the amount of PCV2 shedding in nasal, oral and fecal secretions following natural PCV2 infection of specific pathogen free, commercial swine under controlled conditions.

2. Materials and methods

2.1. Animals and housing

The experimental protocol for this study was approved by the Iowa State University Institutional

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Animal Care and Use Committee (IACUC No. 10-07-6441-S). The pigs were procured from a herd known to be free of PRRSV, SIV and PPV which had recently become PCV2 positive. Four pigs which were PCV2 positive as evidenced by the presence of PCV2 DNA and anti-PCV2-antibodies in serum at 13 days post-farrowing (DPF) were weaned at 3 weeks of age and transported to a BSL-2 animal holding facility at Iowa State University. The pigs originated from 4 litters. The litters were selected based on high amounts of PCV2 DNA in serum at 13 DPF. The dams of these pigs were also PCV2 positive (DNA and antibodies; data not shown). All pigs were housed in the same pen in a single room with a solid concrete floor, a separate ventilation system, and one nipple drinker. The pigs were fed with a balanced, pelleted, complete feed ration free of animal proteins and antibiotics (Nature's Made, Heartland Coop, Iowa, USA) once a day.

2.2. Sample collection

Starting at 28 DPF, nasal swabs, oral swabs, fecal swabs, and serum were collected weekly until termination of the study at 209 DPF. One oral, nasal, and fecal swab were taken per animal using polyester swabs (Fisher Scientific Inc., Pittsburgh, PA, USA). Swabs were stored in 5 ml plastic tubes (Fisher Scientific Inc.) containing 1 ml of sterile saline solution (Fisher Scientific Inc.). Blood was collected in 8.5 ml serum separator tubes (Fisher Scientific Inc.), centrifuged at 2000 × *g* for 10 min at 4 °C. All samples were stored at −80 °C until testing.

2.3. Diagnostic testing

2.3.1. Detection of anti-PCV2-IgG antibodies

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

2.3.2. PCV2 DNA detection and quantification

DNA-extraction was performed using the QIAamp® DNA Mini Kit (Qiagen, Valencia, CA, USA). DNA-extracts were used for quantification of the PCV2 DNA copy numbers by a real-time PCR as previously described using ORF1-based primers (Opriessnig et al., 2003). The PCR reaction consisted of 25 μl PCR mixtures that contained 12.5 μl of commercially available master mix (TaqMan Universal PCR Master Mix, Applied Biosystems Inc., Foster City, CA, USA), 2.5 μl DNA extract, 1 μl forward and reverse primers, 7.5 μl water, and 0.5 μl detection probe with concentrations of 10 μM. On each plate, five progressive 1:10 dilutions of a known copy number of PCV2 DNA excised from a purified PCV2 DNA clone was included to generate a standard curve. Each plate was run in the sequence detection system (7500 Sequence Detection System; Applied Biosystems Inc.) under the following conditions: 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles of 15 sec at 95 °C and 1 min at 60 °C. Samples which did not generate a signal following 40 cycles were considered negative.

2.3.3. PCV2 sequencing

PCV2 sequencing was conducted on all pigs using serum from DPF 13 and DPF 209. A nested PCR was used to amplify the entire ORF2 gene of PCV2 for sequencing as previously described (Opriessnig et al., 2006b). The PCR products were purified using the QIAquick PCR purification kit (Qiagen) per manufacturer's instructions and sequenced at the Iowa State University DNA Sequencing Facility. Sequences were analyzed with Sequence Scanner 1.0 (Applied Biosystems Inc.) and aligned using MegAlign (Lasergene DNAStar version 4.0.43, DNASTAR, Inc., Madison, WI, USA).

2.3.4. PCV2 immunohistochemistry (IHC)

IHC detection of PCV2-specific antigen was performed on selected formalin-fixed and paraffin-embedded sections of lymph nodes (superficial inguinal, mediastinal, tracheobronchial, and mesenteric), tonsil, spleen, Peyer's patches, and thymus using a rabbit polyclonal antiserum (Sorden et al., 1999). PCV2-antigen scoring was done by a pathologist blinded to treatment groups. Scores ranged from 0 (no signal) to 3 (more than 50% of the lymphoid follicles contain cells with PCV2-antigen staining) (Opriessnig et al., 2004a).

2.3.5. Assays to exclude the presence of other potential swine pathogens in the samples

Serum samples collected at initiation and again at termination of the study were tested for the presence of specific antibodies to PRRSV with a commercial PRRSV ELISA (HerdChek® PRRS 2XR Antibody ELISA; IDEXX Laboratories, Inc. Westbrook, MA, USA), for the presence of PPV-specific antibodies by a hemagglutination inhibition (HI) assay (Mengeling et al., 1988), and for the presence of SIV antibodies by an in house nucleoprotein NS1 ELISA (Opriessnig et al., 2010).

2.4. Necropsy

Animals were humanely euthanized with an overdose of pentobarbital (Vortech Pharmaceuticals, Dearborne, MI, USA) and necropsied on 209 DPF. Macroscopic lung lesions, scored from 0 to 100% of the lung affected, and the size of lymph nodes, scored from 0 (normal) to 3 (enlarged four times the normal size), were estimated in a blinded fashion as described previously (Opriessnig et al., 2006a). Sections of lymph nodes (superficial inguinal, mediastinal, tracheobronchial, mesenteric), lung, 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.

2.5. Histopathology

Microscopic lesions were evaluated by a veterinary pathologist (AP). Lung sections were scored for the presence and severity of interstitial pneumonia ranging from 0 (normal) to 4 (severe interstitial pneumonia) as described previously (Halbur et al., 1995). Sections of heart, liver, kidney, ileum, and colon were evaluated for the presence of lymphohistiocytic inflammation, and scored

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