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Research paper

# Markedly different immune responses and virus kinetics in littermates infected with porcine circovirus type 2 or porcine parvovirus type 1

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ABSTRACT

Porcine parvovirus type 1 (PPV1) and porcine circovirus type 2 (PCV2) are small single-stranded DNA viruses with high prevalence in the global pig population. The aim of this study was to compare and contrast PCV2 and PPV1 infections in high-health status pigs and to describe PCV2 long-term infection dynamics. Six caesarian-derived colostrum-deprived pigs were randomly divided into two groups and were experimentally infected with PCV2 or PPV1 at 5 weeks of age. All pigs had detectable viremia by day (D) 3 post-infection. Pigs infected with PPV1 had a detectable INF-α response by D3 followed by a high IFN-γ response by D6. The PPV1 pigs developed antibodies against PPV1 by D6 resulting in decreasing virus titers until PPV1 DNA became undetectable from D28 until D42. In contrast, PCV2-infected pigs had no detectable INF-α or IFN-γ response after PCV2 infection. PCV2-infected pigs had no detectable anti-PCV2 humoral response until D49 and had a sustained high level of PCV2 DNA for the duration of the study. While PPV1-infected pigs were clinically normal, PCV2-infected pigs developed severe clinical illness including fatal systemic porcine circovirus associated disease (PCVAD) by D28, fatal enteric PCVAD by D56 and chronic PCVAD manifested as decreased weight gain and periods of diarrhea. Microscopically, all three PCV2-infected pigs had lymphoid lesions consistent with PCVAD and associated with low (chronic disease) to high (acute disease) levels of PCV2 antigen. Under the study conditions, there was a lack of early IFN-γ and INF-α activation followed by a delayed and low humoral immune response and persisting viremia with PCV2 infection. In contrast, PPV1-infected pigs had IFN-γ and INF-α activation and an effective immune response to the PPV1 infection.

## 1. Introduction

Several small, non-enveloped, single-stranded DNA viruses circulate in pigs including porcine circovirus type 2 (PCV2) and porcine parvovirus type 1 (PPV1). Both of these viruses are highly resistant to inactivation and widespread in pig herds worldwide. While there are several parvovirus species (PPV1 (Dunne et al., 1965), PPV2 (Hijikata et al., 2001), PPV3 (Lau et al., 2008), PPV4 (Cheung et al., 2010), PPV5 (Xiao et al., 2013), PPV6 (Ni et al., 2014), PPV7 (Palinski et al., 2016)), only PPV1 is typically associated with disease. PPV1 was first identified in the 1960s as a cause of reproductive failure in breeding herds (Mengeling et al., 1991) but can also cause cutaneous lesions in growing pigs (Kresse et al., 1985; Lager and Mengeling, 1994). PPV1 has a tropism for actively replicating cells such as fetal myocardiocytes and fetal infection often results in death, which, depending on the stage of pregnancy, can manifest as increased numbers of mummified fetuses, stillborn or weak born pigs. Vaccines to control PPV1 are routinely used in breeding herds.

PCV2 is associated with several different clinical presentations of pigs referred to as PCV2 associated disease or PCVAD. Systemic disease (Opriessnig et al., 2007) or postweaning multisystemic wasting syndrome (Harding, 2007) in growing pigs is the most common PCVAD. PCV2 also plays a role in the porcine respiratory disease complex (Harms et al., 2002; Kim et al., 2003) and has been associated with enteric disease in grow-finish pigs (Jensen et al., 2006; Opriessnig et al., 2011a). An association with porcine dermatitis and nephropathy syndrome (PDNS) is also suspected (Rosell et al., 1999); however, PDNS may occur in pigs free of PCV2 indicating that several factors can cause this disease syndrome. PCV2 strains can be divided into different genotypes of which PCV2a was the most prevalent genotype before 2000 (Fenaux et al., 2000), followed by a global shift towards PCV2b (Patterson and Opriessnig, 2010) which has since been followed by

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another shift towards PCV2d (Xiao et al., 2015). While differences in pathogenicity between PCV2 genotypes are suspected, experimental comparison of strains revealed that strain differences may be more important than genotype differences (Opriessnig et al., 2008; Opriessnig et al., 2014b). Vaccines to control PCV2 are routinely used in growing pigs and less frequently in the breeding herd.

The aim of this study was to generate anti-PPV1 and anti-PCV2 antiserum over time from high health status pigs; however, unique infection kinetics were observed and thus are described in this manuscript.

## 2. Materials and methods

### 2.1. Ethical statement

The experimental protocols were approved by the Iowa State University Institutional Animal Care and Use Committee (Approval number: 1-11-7071-S).

### 2.2. Animals, housing, experimental design and serum collection

Six 4-week-old, caesarian-derived colostrum-deprived (CDCD) male pigs from one litter were obtained from a commercial source (Struve Labs International, Manning, IA, USA), transported to Iowa State University, and randomly divided into two groups and rooms of three pigs. The aim of the study was to generate antibody positive control serum for PCV2 and PPV1 for future evaluation and standardization of serology assays. The experimental design is summarized in Fig. 1. The experimental endpoint of the study was initially day 49 (D49) as antibody levels to PPV1 and PCV2 are usually high at that time. To obtain serum reflecting seroconversion with low to medium levels of antibodies, blood was collected initially every three days until D15 then on D21 and weekly thereafter. Blood was collected in serum separator tubes (Fisher Scientific, Pittsburgh, PA, USA), centrifuged at 3000 × *g* for 10 min at 4 °C and the serum was stored at −80 °C until testing.

### 2.3. Inoculation

At 5 weeks of age, pigs 121, 124 and 125 in the PCV2 group were inoculated using an infectious PCV2a stock (strain 40895; Fenaux et al., 2003) with a titer of $10^{4.5}$ 50% tissue culture infectious dose ($TCID_{50}$) per ml intramuscularly (1 ml) and intransally (1 ml). For the PPV1 inoculation, a frozen tissue stock inoculum (NADC, Ames, Iowa) was used as described (Opriessnig et al., 2011b). In brief, PPV isolate NADL-8 was isolated in fetal porcine kidney cells from a naturally infected pig in 1977 (Mengeling, 1979) and was subsequently passaged in fetuses in the pregnant sow model. Passage 4 of the PPV challenge virus stock was used at an approximate titer of $10^{4.9}$ $TCID_{50}$ per ml (Opriessnig et al., 2011b). Each pig in the PPV1 group (pigs 99, 122 and 123) received 1 ml of the PPV1 tissue homogenate intranasally by slowly dripping 0.5 ml into each nostril.

### 2.4. Clinical observations

All animals were examined daily for signs of illness such as lethargy, respiratory disease, diarrhea, inappetence and lameness.

### 2.5. Serology

All serum samples were tested for the presence of PCV2 IgG by an *in house* indirect ORF2-based ELISA (Nawagitgul et al., 2002). Samples were considered positive if the sample-to-positive (S/P) ratio was equal to or greater than 0.2 (Nawagitgul et al., 2002). In addition, all samples were tested by a commercial blocking whole PCV2-based anti-PCV2 IgG ELISA (SERELISA® PCV2 Ab Mono Blocking; Zoetis). Sample titers were calculated based on single dilutions using the calculation sheet supplied by the manufacturer. To determine the presence of PPV1 antibodies, all serum samples were tested by a commercial blocking ELISA (Ingezim PPV Compac; R.11.PPV.K3; Ingenasa, Madrid, Spain). This assay is based on recombinant VP2 PPV1 protein. Samples with a blocking percentage > 30% are considered positive, samples < 25% are considered negative, and samples with a blocking percentage from 25 to 30% are considered indeterminate and could be positive or negative. Serum samples collected at D21 and D35 were also tested for the presence of PPV1 antibodies by a hemagglutination inhibition (HI) assay as described (Mengeling et al., 1988).

### 2.6. DNA extraction, detection and quantification amount of PCV2 and PPV1

Total nucleic acids were extracted from all serum samples using the MagMax™ Pathogen RNA Kit (Applied Biosystems, Life Technologies, Carlsbad, CA, USA) on an automated nucleic acid extraction system (Thermo Scientific Kingfisher® Flex, Thermo Fisher Scientific, Pittsburgh, PA, USA) according to the instructions of the manufacturer. Negative and positive controls were included in each run. All DNA extracts were tested for the presence of PCV2 with an ORF1-based real-time PCR assay as described (Opriessnig et al., 2003). In addition, all DNA extracts were tested for the presence of PPV1 by a quantitative PPV1 real-time PCR assay targeting the VP2 region (Opriessnig et al., 2011b). For both assays, samples were considered negative when no signal was observed by a cycle-threshold ($C_T$) of 40. To further characterize the PCV2 strain present in the pigs, selected PCV2 DNA positive serum samples were tested by a PCV2a/2b/2d differential real-time PCR assay targeting the ORF2 (Opriessnig et al., 2010a; Opriessnig et al., 2014a). Selected PCV2 DNA positive samples were also subjected

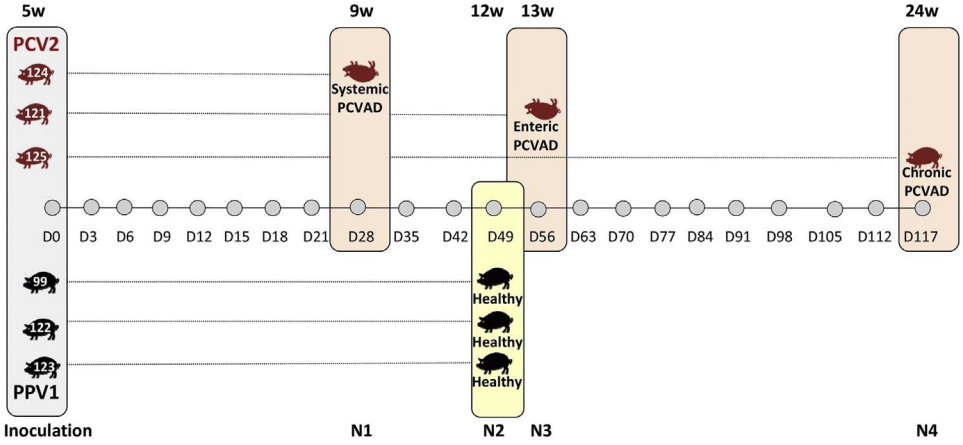


**Fig. 1.** Experimental design. Blood samples were collected on certain days indicated by grey circles. The age of the pigs in weeks is indicated on top. N = necropsy.