RESEARCH REPORT
SWINE HEALTH

Title: Characterization of porcine parvovirus type 3 (PPV3) infection in growing pigs – **NPB #12-187**

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Date Submitted: February 17, 2014

Industry Summary:

In recent years increasing usage of molecular biology tools has resulted in identification of a variety of previously unknown virus species. Porcine parvovirus type 3 (PPV3) is considered an emerging pathogen in pigs although little information on its distribution and pathological potential is available. The objective of this study was to further characterize PPV3 infection in pigs. A serology assay to detect a specific antibody response to PPV3 was developed and will be a useful tool to further conduct epidemiological investigations. In addition, an infectious clone was constructed which also will be useful for future investigations. The obtained *in vivo* results after experimental infection of CDCD pigs indicate that PPV3 viremia is rather short and of low magnitude in healthy pigs. Furthermore, seroconversion occurred after 2 weeks which is similar to other pathogens in pigs such as classical PPV and porcine circovirus type 2 (PCV2).

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Keywords: Porcine parvovirus type 3; serology; viremia; pig model.

Scientific Abstract

In recent years increasing usage of molecular biology tools has resulted in identification of a variety of previously unknown virus species. Porcine parvovirus type 3 (PPV3) is considered an emerging pathogen in pigs although little information on its distribution and pathological potential is available. The objective of this study was to further characterize PPV3 infection in pigs. A serology assay to detect a specific antibody response to PPV3 was developed and will be a useful tool to further conduct epidemiological investigations. In addition, an infectious clone was constructed which also will be useful for future investigations. The obtained *in vivo* results after experimental infection of CDCD pigs indicate that PPV3 viremia is rather short and of low magnitude in healthy pigs. Furthermore, seroconversion occurred after 2 weeks which is similar to other pathogens in pigs such as classical PPV and porcine circovirus type 2 (PCV2).

Introduction

Parvoviruses are ubiquitous and are associated with a broad spectrum of clinical disease in animals including reproductive failure, enteritis, panleukopenia, hepatitis, erythrocyte aplasia, immune complex-mediated vasculitis, and cerebellar ataxia (Claude et al., 2004; Mengeling et al., 2000). In 2008, a novel parvovirus, Hokovirus, recently renamed as partetravirus (PtV) and now known as PPV3 (Xiao et al., 2013), was discovered in samples obtained from domestic pigs and cattle in Hong Kong. PPV3 was later also identified in domestic pigs in the United Kingdom and the wild boar populations in Germany and Romania (Adlhoch et al., 2010; Cadar et al., 2011; Lau et al., 2008; Szelei et al., 2010).

The family *Parvoviridae,* subfamily Parvovirinae, is further divided into five genera: Parvovirus, Erythrovirus, Dependovirus, Amdovirus and Bocavirus (Tattersall et al., 2005). Parvoviruses are small, non-enveloped, single-stranded DNA viruses, with a genome size of approximately 4,000 to 6,000 nucleotides that contain terminal palindromic sequences (Cheng et al., 2010; Claude et al., 2004).

PPV3 contains a single-stranded DNA genome of approximately 5 kb and the genome has two ORFs coding for non-structural and capsid proteins. PPV3 differs from other parvoviruses by its relatively large predicted VP1 protein and the presence of a unique small putative protein (Lau et al., 2008). Molecular analysis of bovine PtV (BPtV) and PPV3 confirmed that these viruses are highly related to human PtV (HPtV) also known as human PARV4 with nucleotide identities of 61.5 to 63.0%. Humane PARV4 was initially identified in 2005 in the plasma of a hepatitis B positive intravenous drug user with an acute viral infection (Jones et al., 2005) and was subsequently detected in plasma from AIDS patients and hepatitis C virus infected persons. Human PARV4 was further determined to have at least three genotypes: genotype 1, genotype 2 (previously termed PARV5) and genotype 3 (Fryer et al., 2007; Longhi et al., 2007; Panning et al., 2010; Simmonds et al., 2008). Interestingly, human PARV4 can be frequently detected in human coagulation factor concentrates prepared from older plasma samples and is suspected to be potential cause of encephalitis in children (Benjamin et al., 2011; Schneider et al., 2008). Recently, ovine PtV (OPtV) and an additional genotype of BPtV were identified (Tse et al., 2011). PPV3 and PtVs are currently regarded as a separate genus in the *Parvovirinae* (Tse et al., 2011).

In domestic pigs in Hong Kong, the overall prevalence of PPV3 was revealed to be 44.4% (148/333) based on testing of lymph nodes, livers, serum samples, nasopharyngeal swabs and feces (Lau et al., 2008). Similarly, the prevalence of PPV3 in Germany, based on testing of liver and serum samples obtained from wild boars, was 32.7% (Adlhoch et al., 2010). Recently, PPV3 prevalence rates ranging from 22.8% to 50.5% were identified in samples of lymph nodes, lungs, liver, kidneys, spleen and tonsils collected from wild boars in Romania in 2006 through 2007 or in 2010 through 2011, respectively (Cadar et al., 2011).

Objectives

The objective of this study was to characterize the PPV3 infection in growing pigs including pathogenicity, viremia and antigenic properties.

Materials and Methods

A. Isolation of PPV3

Virus isolation on PCR positive tissues was conducted on primary porcine kidney cells utilizing randomly selected PPV3 DNA positive samples obtained through the ISU-VDL.

B. Experimental infection of pigs with PPV3

Caesarian-derived, colostrum deprived pigs and conventional high-health-status pigs negative for PPV by serology and PCR were utilized. The animals were randomly divided into different groups and rooms. Inoculation varied by experiment and is described in the results. Blood and fecal swabs were collected on a regular basis and tested for presence and amount of PPV3 DNA and antibody. Necropsy was conducted at 23 days post inoculation.

C. Molecular analysis

All tissues were tested by quantitative real-time PCR assays for PPV3 DNA developed by our group (Xiao *et al*., 2013).

D. Serology

To develop a serological method for detection of anti-PPV3 antibodies, two pairs of primers were designed to express fragments within VP1 protein, which were predicted to have antigenic peptides by software DNASTAR and DNAMAN. The fragments were 100 and 98 amino acids in length. The recombinant plasmids were confirmed by restriction digestion and sequencing, and the proteins were confirmed by SDS-PAGE. Subsequently an ELISA was developed and the assay was tested for cross-reactivity with PPV1, PPV2, PPV4 and PPV5 and appears to be specific and sensitive. The assay was validated on pigs experimentally infected with PPV3.

E. Infectious clone production

The main part of the PPV3 genome was amplified by primers designed based on the known partial genome sequences from GenBank. To obtain the 5' and 3' termini of the genome, ploy Gs were added to the viral genomic DNA ends and then the ends were cloned and sequenced. To construct the infectious clone of PPV3, the whole genome was sequentially cloned by three overlapping fragments into pMD19 vector with restriction sites introduced, and the clone is designed as pMD-PPV3 (Fig. 1). After confirmation by restriction digestion and sequencing, the full genome of PPV3 was further cut from the pMD19 vector and then cloned into the pCI-neo mammalian expression vector, which was named as pCI-PPV3. pCI-PPV3 was further confirmed by restriction digestion and sequencing . Both clones were used for cell culture and to infect pigs.

Results

Isolation of PPV3

Selected PPV3 DNA positive serum samples were utilized. Virus isolation was conducted on porcine primary fetal kidney cells. The results are summarized in Table 1.

Table 1: PPV3 PCR levels (ct values) in tissue culture during serial passage.

Sample ID	PPV3 ct in the original sample	P2	P3	P4	P5
266	21.3	33.1	33.9	37.5	Negative
270	0	31.7	34.7	Negative	Negative
285	17.2	28.1	30.9	35.5	Negative
320	16.82	24.4	30.2	30.9	Negative
371	15.8	Not tested	Not tested	29.0	Negative
372	24.1	Not tested	Not tested	Negative	Negative
373	18.1	Not tested	Not tested	37.0	Negative
380	26.6	Not tested	Not tested	Negative	Negative

Infectious clone production

The schematic cloning attempt is summarized in Fig. 1. In brief, the PPV3 genome was rescued and cloned into a pMD vector and subsequently transferred into a PCl vector.

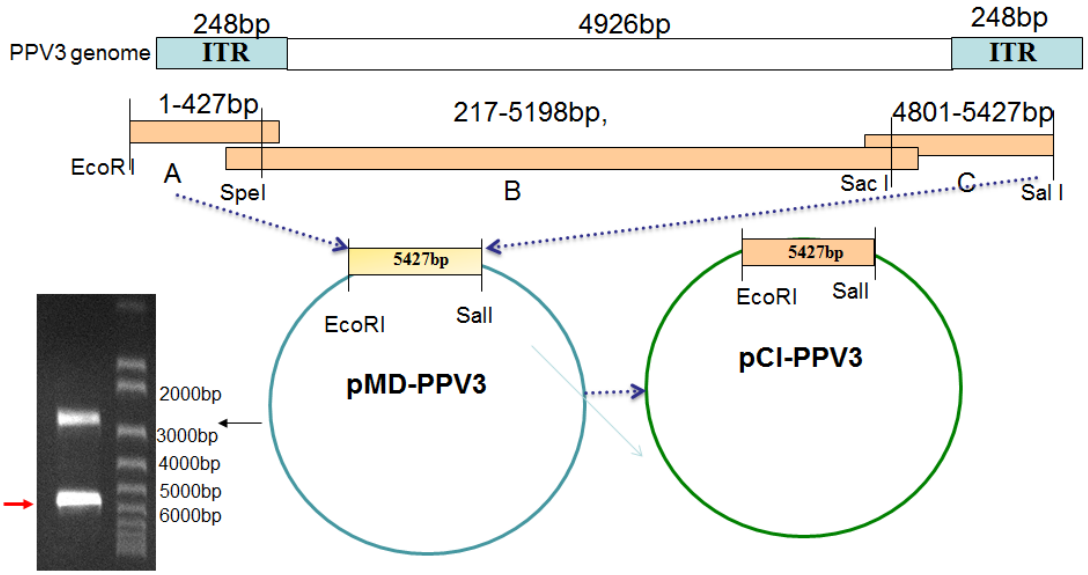

Fig. 1. Cloning strategy for PPV3.

Serology

Initially, a protein was expressed and tested on field samples for cross-reactivity with other known PPVs. Results on selected samples are summarized in Table 2.

Table 2: ELISA OD values for different PPV proteins on selected field serum samples

Sample	PPV1 OD	PPV2 OD	PPV3 OD	PPV4 OD	PPV5 OD
A	1.105	0.663	0.410	0.343	0.380
B	0.234	0.855	0.44	0.15	0.19
C	0.351	1.363	0.437	0.256	0.519
D	0.485	0.485	1.727	0.575	0.797
E	0.567	2.045	1.029	1.520	0.299
F	0.537	0.640	0.422	0.568	1.518

Experimental infection of pigs with PPV3

A. *CDCD pigs*. Four 5-week-old CDCD pigs were randomly assigned to one of two rooms with two pigs in each room. In each room one pig served as non-infected contact control and the other pig was infected with PPV3 DNA positive serum obtained from the field. Inoculation was done by a combination of the intramuscular (1 ml) and the intralymphoid (0.5 ml) routes. Blood and serum samples were collected on days 0, 3, 7, 10, 15, 20 and 23. All pigs were re-challenged on day 15. The sera used for inoculation were determined to be positive for PPV3 by real-time PCR (Ct 22.8 and 28.8) and were negative for PCV2, PPV1, PPV2, PPV3 and PPV5. As indicated in Fig. 2, PPV3 infected pigs seroconverted to PPV3 around 15 days post infection. PCR results indicated that one contact control was weak PCR positive 15 and 20 days post challenge and in the other group the infected pig was weak PCR positive 20 days post challenge. PPV1, 2, 4 and 5 DNA or PCV2 DNA was not identified in any of the pigs. The pigs remaining clinically healthy for the duration of the study and lesions were not observed in any of the pigs.

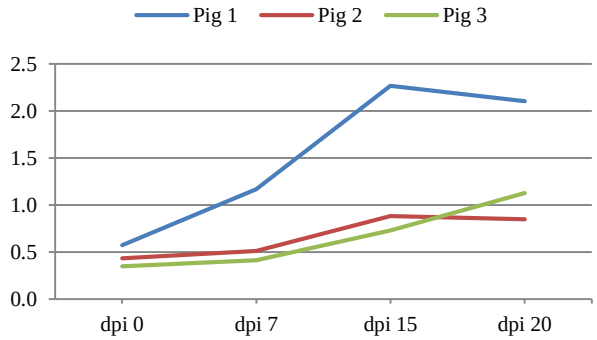

Fig. 2. Serological profile in pigs experimentally infected with PPV3 DNA positive serum at different days post infection.

B. *Conventional pigs*. Two conventional pigs free of PPV3 were infected intramuscularly and intralymphoid with 1 ml containing PPV3 infectious clone passage 1 and 2 (cts 20-26). No viremia was detected by 14 days and none of the pigs had seroconverted at that time.

Discussion

PPV3 is among a number of pig viruses recently identified in SE Asia and other parts of the world. Very little is known about this virus in the U.S. Knowledge of PPV3 in the U.S. pig population was advanced through progress on virus isolation techniques, construction of infectious clones, development of PPV3 specific serology and experimental infection of pigs.

It proved to be much more difficult to propagate PPV3 *in vitro* compared to classical PPV1. Many cell lines and cell culture conditions were utilized in an effort to optimize a protocol that could consistently be used to isolate PPV3. Although we were successful in growing PPV3 in cell culture, PPV3 DNA levels decreased with passage in cell culture and evidence of growth was lost after 3 to 4 passages. Similarly, PPV3 infectious clone material did not result in a recognizable viremia in experimentally infected pigs. However, when pigs

were infected with serum containing PCV2 DNA from a field case, low viremia levels and seroconversion were observed in a portion of the pigs. The low amount of PPV3 DNA is suggestive that PPV3 is likely not pathogenic in pigs by itself; however, it may be similar to the situation with PCV2 where another trigger or confection is necessary for manifestation of PPV3-associated disease in otherwise healthy pigs. Additional research is needed to further understand the role, if any, of PPV3 in common coinfections of growing pigs.

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