

The occurrence of porcine circovirus 3 without clinical infection signs in Shandong Province

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Funding information

The study was partly supported by National Key R&D Program (2016YFD0500708); Shandong Province Modern Agricultural Industry Technology System (SDAIT-08-07); Shandong Province Science and Technology Development Plan (2014GNC111011); Shandong Province Agricultural Applications of Major Innovation Projects; Young Talents Training Program of Shandong Academy of Agricultural Sciences; Taishan Scholars Project; and the Youth Scientific Research Foundation of Shandong Academy of Agricultural Sciences (2014QNM40, 2016YQN53).

Shuxuan Zheng and Xiaoyan Wu are parallel first authors.

Summary

Porcine circovirus type 3 (PCV3) was detected in Shandong, China. One hundred and thirty-two of 222 (59.46%) samples were PCV3 positive, while 52 of 132 (39.39%) samples were co-infected with PCV2. There were no clinical signs of infection in either multiparous sows or live-born infants. Two strains of PCV3 were indentified from natural stillborn foetuses. Phylogenetic analysis showed the two strains of PCV3 are 96% identical to the known PCV3/Pig/USA (KX778720.1, KX966193.1 and KX898030.1) and closely related to Barbel Circovirus. Further studies of the epidemiology of PCV3 and the co-infection with PCV2 are needed.

KEYWORDS

China, co-infection, PCV3, porcine circovirus, swine

1 | INTRODUCTION

Porcine circovirus (PCV) is the first and smallest animal virus shown to possess a circular, single-stranded DNA genome that replicates autonomously in mammalian cells (Tischer, Gelderblom, & Vettermann, 1982). PCV1 and PCV2 are the two basic genotypes of PCV. PCV1 is a cell culture-derived virus and is considered non-pathogenic for swine. PCV2 is the primary causative agent of porcine circovirus-associated diseases (PCVAD) in swine; PCVAD has been referred to by different names, the most common being postweaning multisystemic wasting syndrome (PMWS). Furthermore, porcine dermatitis and nephropathy syndrome (PDNS), proliferative and necrotizing pneumonia, respiratory disease and enteritis were also linked to PCV2 infections (Allan et al., 2000; Madson et al., 2009; Grau-Roma & Segalés, 2007; Cheng et al., 2011; Kim & Chae, 2004) and have caused a huge economic loss to the pig industry worldwide. PCV3, a novel genotype of PCV, was first reported in pigs with cardiac and multisystemic inflammation in 2016 (Phan et al., 2016). About the same time, pigs were detected having PCV3 with PDNS, and reproductive failure was reported (Palinski et al., 2016).

The PCV2 genome is composed of 1,767–1,768 nucleotides and predicted to possess eleven open reading frames (ORFs). The intergenic region (IR) contains the origin of replication with a stem-loop (SL) structure, which includes an octanucleotide sequence (Oc8) flanked by palindromes (Faurez, Dory, Grasland, & Jestin, 2009). Two major ORFs, ORF1 and ORF2, encode replicase (Rep) and capsid protein (Cap), respectively. Cap contains one putative N-glycosylation site at position 143–145aa (NYS) (Nawagitgul et al., 2002), while

Rep contains three potential glycosylation sites at positions 23–25aa (NPS), 256–258aa (NQT) and 286–288aa (NAT) (Mankertz, Mankertz, Wolf, & Buhk, 1998). Regarding clinical aspects, PCV2 is ubiquitous, having both domestic and feral swine hosts; most pigs become infected at 4–11 weeks of age. The common presenting clinical signs of PMWS include wasting or unthriftiness, dyspnoea and visibly enlarged lymph nodes (Segales, Allan, & Domingo, 2005). Characteristic PDNS symptoms are irregular, red-to-purple macules and papules in the skin; subcutaneous haemorrhages and oedema of affected areas; enlarged lymph nodes, mainly inguinal superficial; cutaneous scars in animals that have recovered from the acute phase; bilaterally enlarged kidneys; small cortical petechiae and oedema of the renal pelvis; and occasional spleen infarcts (Segalés, 2012).

With the world's highest pork consumption (accounting for almost 50%), China plays an important role in global pig production. In particular, Shandong is an agriculturally advanced province of China, with a great variety of large-scale pig farms.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Two hundred and twenty-two tissue samples (including hearts, livers, lungs, kidneys, spleens and umbilical cords) were collected from 37 natural stillborn foetuses from seven main, large pig farms (Weihai, Yantai, Linyi, Binzhou, Weifang, Laiwu and Liaocheng) in Shandong Province (Figure 1). No clinical infection signs appeared in either



FIGURE 1 Geographical distribution of porcine circovirus type 3 (PCV3) in eastern China. Red stars represent the PCV3-positive pig farms [Colour figure can be viewed at wileyonlinelibrary.com]

sows or live-born infants. The tissue homogenate was prepared for the regular detection of common porcine viruses (PCV1, PCV2, PPV, PRV, TTV1, TTV2, PRRSV and CSFV) using PCR.

2.2 | Viral metagenomics

The viral metagenomic deep sequencing was performed by MiSeq Reagent Kit V2 (Illumina).

2.3 | Phylogenetic analysis

The complete genomes of two isolates were sequenced and compared with 22 complete sequences of the *Circoviridae* family isolates from twelve different countries. The Cap genome of the two isolates were sequenced and compared with fifteen Cap sequences of the *Circoviridae* family from eight different countries, and the Rep genome of the two isolates were sequenced and compared with seventeen Rep sequences of the *Circoviridae* family from twelve

different countries. Phylogenetic analysis was performed using MEGA 6.0.

2.4 | Genetic analysis

The genetic analysis was performed using BLASTN and BLASTP (NCBI).

3 | RESULTS AND DISCUSSION

Interestingly, most of the tissues tested negative for PCV2. The viral metagenomic deep sequencing showed the possibility of the presence of a novel virus. PCV3 was identified by routine PCR with the forward primer 5'-CCACAGAAGGCGCTATGTC-3' and reverse primer 5'-CCGCATAAGGGTCGTCTTG-3'. PCR products were verified by Sanger sequencing. Two strains were identified as PCV3 and were designated as PCV3/Pig/CN/Shandong-1/2017 and PCV3/Pig/CN/Shandong-2/2017.

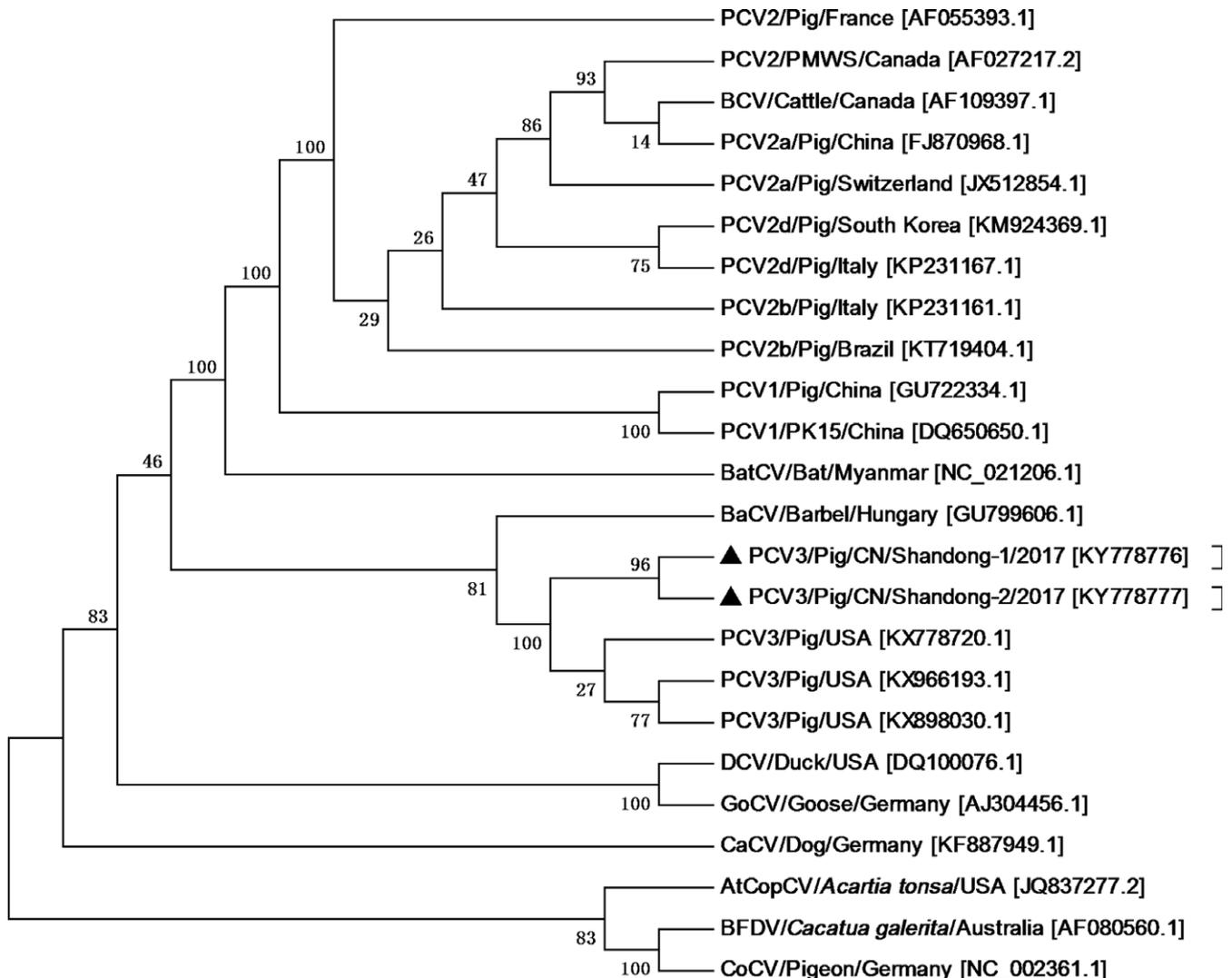


FIGURE 2 Phylogenetic tree of the complete sequences of PCV3. Phylogenetic tree was constructed using the maximum-likelihood algorithm of MEGA 6.0 with 1,000 bootstrap trials

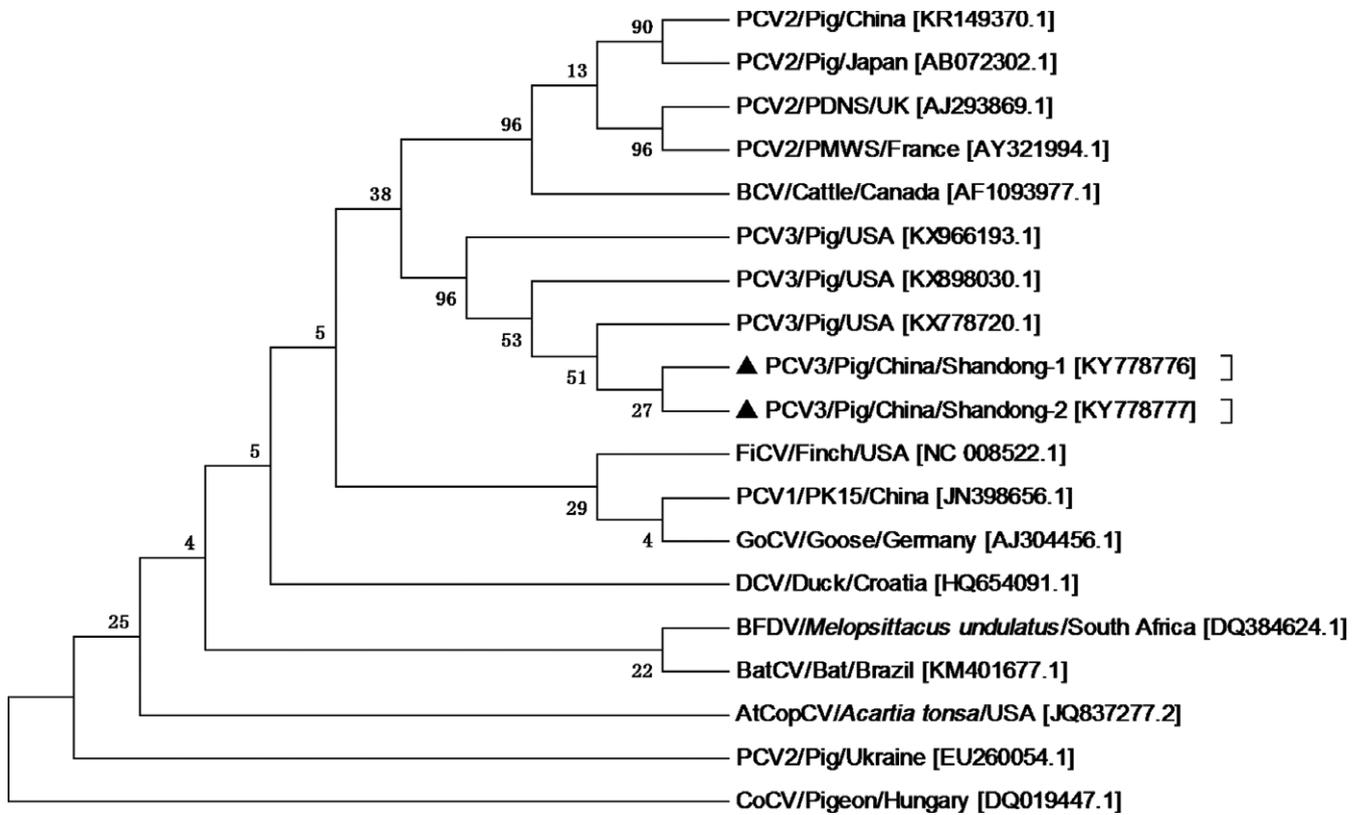


FIGURE 3 Phylogenetic tree of the Rep gene of PCV3. Phylogenetic tree was constructed using the maximum-likelihood algorithm of MEGA 6.0 with 1,000 bootstrap trials

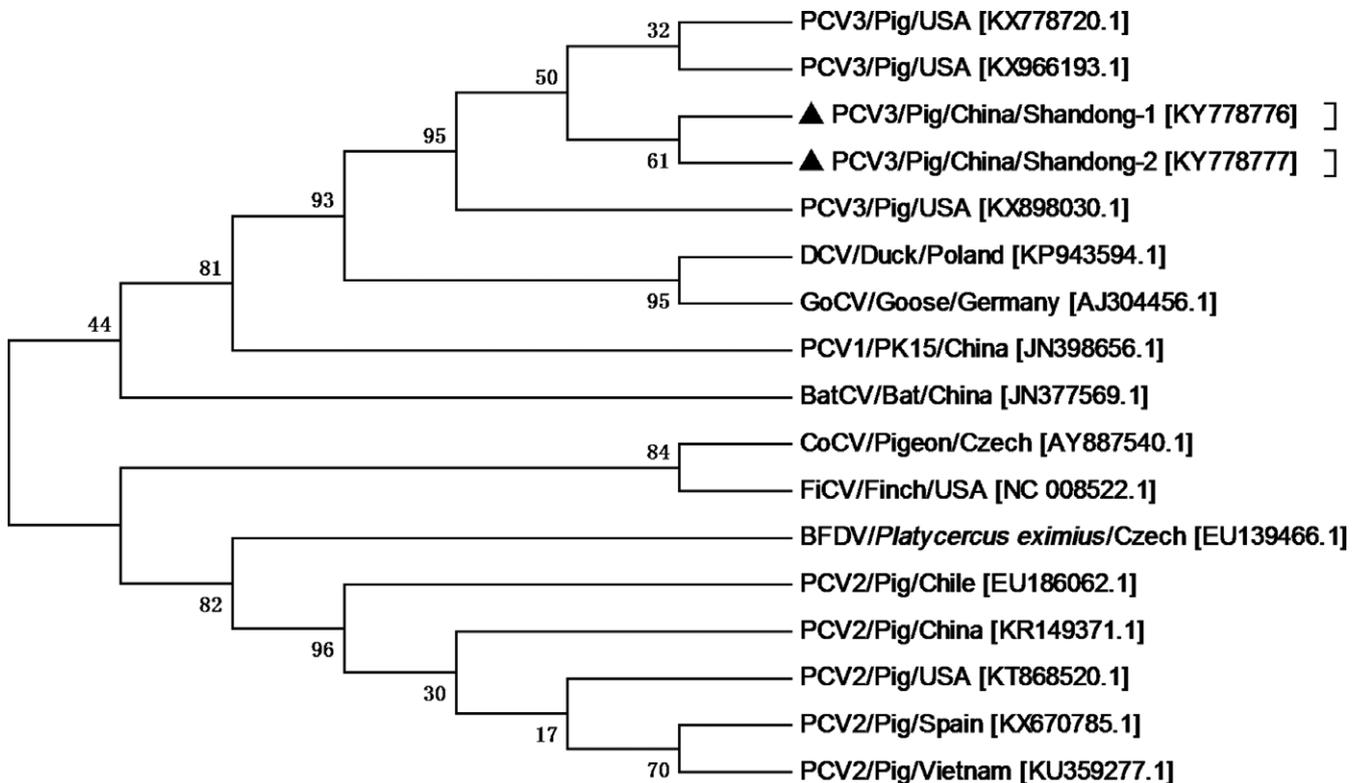


FIGURE 4 Phylogenetic tree of the Cap gene of PCV3. Phylogenetic tree was constructed using the maximum-likelihood algorithm of MEGA 6.0 with 1,000 bootstrap trials

One hundred and thirty-two tissue samples (livers, lungs, kidneys, spleens and umbilical cords) of 222 samples (hearts, livers, lungs, kidneys, spleens and umbilical cords) tested positive for PCV3. Furthermore, 52 of 132 samples tested positive for PCV2. Nucleotide identity analysis of the two isolates revealed that both viruses were homologous with each other, sharing 99.0%–100.0% nucleotide identity. The PCV3 viral genome contains 2,000 bases with two major ORFs encoding Cap and Rep. According to proteomic analyses, PCV3 has one phosphorylated threonine at position aa 444 (T), one predicated N-glycosylation site at aa 191 (191aa NLT) and five O-linked glycosylation sites at aa 13, 403, 410, 414 and 416. The phylogeny of PCV3 complete sequences indicated that the two isolates are 96% identical to the known PCV3/Pig/USA (KX778720.1, KX966193.1 and KX898030.1) and closely related to Barbel Circovirus (BaCV/Barbel/Hungary, GU799606.1) (Figure 2). Phylogenetic analysis of Rep gene also showed the highest identity with the known PCV3 Rep sequences (Figure 3). In addition, this Rep might share an ancestor in common with Bovine coronavirus (BCV/Cattle/Canada, AF1093977.1) in a clade containing PCV2 (KR149370.1, AB072302.1, AJ293869.1 and AY321944.1). The Cap protein showed high nt identity with the known PCV3 (Figure 4).

All PCV3-infected pigs in this study were naturally stillborn foetuses. According to previous reports, PCV3 is associated with PDNS, reproductive failure, cardiac and multisystemic inflammation. Interestingly, there is no clinical infection sign of either PCV3-associated disease or PMWS shown in sows and live-born in this study. The chronicles of each pig farms were queried to determine the previously mentioned outcome. One of the seven large-scale pig farms (Yantai) was found to have had introduced breeds from France, and the sows were injected with the vaccine from Boehringer Ingelheim. However, to the best of our knowledge, PCV3 has not yet been reported to date in France or Europe. Further study should focus on the epidemiology of PCV3 and the co-infection of PCV2-PCV3.

3.1 | Nucleotide sequence accession numbers

The GenBank accession numbers of PCV3/CN/Shandong-1/2017 and PCV3/CN/Shandong-2/2017 are KY778776 and KY778777.

ACKNOWLEDGEMENT

We gratefully acknowledge Douglas M. Eubanks for English language editing.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AVAILABILITY OF DATA AND MATERIALS

The data set supporting the conclusions of this article is available in the GenBank.

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How to cite this article: Zheng S, Wu X, Zhang L, et al. The occurrence of porcine circovirus 3 without clinical infection signs in Shandong Province. *Transbound Emerg Dis*. 2017;64:1337–1341. <https://doi.org/10.1111/tbed.12667>