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SHORT COMMUNICATION

Prevalence and Genetic Characterization of Porcine Circovirus Type 3 in Guangxi Province from 2009 to 2017

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ABSTRACT

Porcine circovirus 3 (PCV3) is a novel porcine circovirus type recently reported in many countries. PCV3 has suspected links to porcine dermatitis and nephropathy syndrome (PDNS). In this study, 843 tissue and serum samples of pigs collected between 2009 and 2017 in Guangxi province of China were tested for PCV3. The tissue and serum positive rate of PCV3 was 7.44 and 2.96%, respectively. PCV3 could be detected from different tissues of pigs showed that it has wide tissue tropism. Fifteen complete genome sequences of PCV3 were obtained and showed high genetic identity with other PCV3 isolates. A recombinant cluster had been found based on the phylogenetic tree analyses and similarity plot analyses of ORF2. Our study identified PCV3 was found widely spread in the Guangxi region since 2009. Genomic recombination in different clusters of PCV3 indicated the possible genetic and molecular evolution in the virus.

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INTRODUCTION

Porcine Circovirus (PCV) is a non-enveloped circovirus containing a ~1.7 kb single-stranded circular DNA genome (Ellis, 2014). PCVs are classified in the genus *Circovirus* within the family *Circoviridae*, porcine circovirus type 1 (PCV1) and porcine circovirus type 2 (PCV2) are two circovirus species have been described as infectious in pigs according to the pathogenicity, antigenicity and nucleotide sequence of PCVs (Taeyong *et al*., 2017), PCV2 infection in pig causes a serious clinical disease known as Porcine Circovirus Associated Disease (PCVAD). PCVs contain two major open reading frames: ORF1 codes for the replicase protein (Rep) and ORF2 for the capsid protein (Cap) (Meng, 2013). The capsid protein of circovirus is the major structural protein and it possesses the antigenic characteristics of the virus (Nawagitgul *et al.*, 2000; Fu *et al*., 2017).

In 2016, another porcine circovirus species was identified called PCV3. The PCV3 was detected in suckling piglets and found associated with porcine dermatitis, nephropathy syndrome (PDNS) and reproductive failure in different parts of US (Phan *et al.*, 2016; Palinski *et al.*, 2017). The full-length genome sequence of PCV3 has 2000 nucleotides, and the capsid and replicase proteins of PCV3 are shared only 37% and 55% identical, respectively, to PCV2 (Rachel *et al.*, 2016; Ku *et al.*, 2017). In addition, PCV3 was detected in different countries in the world. In China, PCV3 was detected in piglets with reproductive failure and respiratory disease, and it was closely associated with PCV2 (Zhai *et al.*, 2017). However, the origin, prevalence, tissue tropism and role in the diseases of PCV3 are unknown. Therefore, objective of our study was to identify the prevalence and genetic characteristics of PCV3 in serum and tissue samples of pigs collected between 2009 and 2017 in Guangxi province of China.

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MATERIALS AND METHODS

The prevalence of PCV3 was detected in 843 samples (336 tissues and 507 serums) which were collected from 13 pig farms in Nanning, Qinzhou, Hepu, Wuzhou, Linshan, Yulin, Guilin, Laibin, Hezhou, Liuzhou, Guigang, Fangchengang and Dongxin, and four slaughterhouses in Guigang, Guipin and Wuxuan in Guangxi province. All samples were stored at -80°C until used in genetic analysis. Another 280 samples preserved at -20°C between 2009 and 2016 were also included in the analysis.

Viral DNA was extracted from tissue lysates and serum samples by using a commercial DNA/RNA extraction kit (Axygen, Corning, NYC, NY). Primers set targeted to amplify 649 bp of PCV3 ORF2 were designed (Ku *et al.*, 2017). PCR was performed using Green Taq Mix (Vazyme Biotech, Nanjing, China) and the reaction condition was pre-denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 30s, annealing at 50°C for 30s and extension at 72°C for 30s and a final extension at 72°C for 10min. The complete genome was amplified using three pairs of primers including the detection primers (Table 1). The amplicons of the complete genome were gel-purified using a gel extraction kit (Omega Bio-tek, Norcross, GA, USA), cloned into pMD18-T Vector (Takara Bio, Kusatsu, Japan) and sequenced (Beijing Genomics Institute, Guangzhou, China).

Complete genome sequences of 27 PCV3 isolates were downloaded from the GenBank. Multiple sequence alignments were performed with MEGA 6.06 software. The genetic identities of these isolates were analyzed with the Clustal W method in the Lasergene software package (DNAStar Inc., Madison, WI). The phylogenetic trees were generated using the maximum likelihood method based on the General Time Reversible model (bootstrap analysis with 1,000 replicates). The genetic distance of ORF2 was calculated with the p-distance method (bootstrap analysis with 1,000 replicates) by using MEGA 6.06. Similarity plot analyses of PCV3 ORF2 were performed by the maximum likelihood distance model in SimPlot v3.5.1.

RESULTS AND DISCUSSION

The total PCV3 positive samples collected between 2009 and 2017 was 4.74% (40/843). The PCV3 positive tissue and serum samples separately were 7.44% (25/336) and 2.96% (15/507), respectively. The samples positive for both PCV3 with PCV2 was 37.5% (15/40). When we split the prevalence year-wise, we noticed that PCV3 positive samples between 2009 and 2015 was 1.4% (3/214); 2016 was 3.03% (2/66); and 2017 was 6.21% (35/563), suggesting an alarming increase over the last few years. In 2017, PCV3 positive samples collected exclusively from 12 regions in the Guangxi province swine farms and slaughter houses was very high with 60% (15/25). PCV3 positive samples were detected in tissues like lungs, liver, lymph nodes, kidney, spleen and intestines. In addition, the incidence of co-infections rate of PCV3 with PCV2 was 37.5% (15/40). Details of types of tissues and co-infection status of the PCV3 positive samples are not shown.

The complete genome sequence of 15 PCV3 isolates had 2000 nucleotides (nt) in length. In particular, 3 out of the 15 PCV3 isolates were from samples collected in 2009 (GXYL-2009-1; GXYL-2009-2; GXYL-2009-3), 1 isolate in 2016 (GXNN-2016-4) and 8 isolate in 2017 (GXGG-2017-5; GXGP-2017-6; GXFC-2017-7; GXFC-2017-8; GXFC-2017-9; GXFC-2017-10; GXFC-2017-11; GXFC-2017-12). The putative ORF1 (Rap) and ORF2 (Cap) were 888 nt and 645 nt long, respectively, which were identical in size compare with other PCV3 isolates. Our 15 PCV3 isolates shared 98.5-99.8 and 97.5-99.8% homology in the genome sequence of ORF1 and ORF2, respectively. A comparison of the complete sequence of 27 PCV3 isolates available in the GenBank database with our 15 new isolates indicated that ORF1 and ORF2 sequence similarity was ranged from 98.6-99.3 and 97.5-99.5%, respectively. The ML phylogenetic tree analyses based on the complete genome of the ORF2 are shown in Fig. 1.

Our study results of PCV3 prevalence during 2009 to 2017 suggested that the virus was present much before 2009, and PCV3 has close relationship with PCV2 associated disease. The percent positive prevalence rate of PCV3 from 2009 onwards represents an alarming increase from the last few years. PCV3 could be detected from different tissues of pigs showed that it has wide tissue tropism. PCV3 infected pigs exhibit the clinical manifestations of PMWS and reproductive failure (Phan *et al.*, 2016; Panlinski *et al.*, 2017). We also detected PCV3 in the lymph nodes of apparently healthy slaughtered pigs, indicating that PCV3 infections tend to be asymptomatic (Stadejek *et al.*, 2017; Zheng *et al.*, 2017). The consequence of asymptomatic PCV3 cases on the viral epidemiology and transmission remains unclear.

Phylogenetic tree analyses based on the ORF2 divided the PCV3 into two mainly distinct clusters: PCV3a and PCV3b. The genetic distance between these two clusters are greater than 0.012. Interestingly, sequence analysis of the ORF2 of the undefined cluster of PCV3 isolates showed the evidence of possible genomic recombination events happening in the virus. When we set the GXGP2017-6 ORF2 sequence belongs to undefined cluster as a query sequence we found its parental sequences were KX458235.1 PCV3/2164/USA/2015/ CAP and KY075986 PCV3/Fujian-5/China/2016 which belonged to PCV3b and PCV3a, respectively. Similar results were observed by Simplot and bootscan analyses for the undefined clusters of other PCV3 isolates (Fig. 2a, b). When the genomic distances of ORF2 of PCV2 and PCV3 genotypes were compared, the PCV3 sequence was found highly homologous and more conservative than PCV2 (Hesse *et al.*, 2008). We divided PCV3 genotypes based on the complete genetic distance between sequences by phylogenetic tree and ORF2 and found it greater than 0.012. Two different genotypes of PCV3 (PCV3a and PCV3b) were isolated from 2009 samples suggesting its possible prevalence before 2009 and recombination event is characteristic of PCV3 in the genetic evolution.

Table 1: Primer sequences used in this study

Primer name	Nucleotide sequence	Product size	Purpose
PCV3-F	ACTTAGAGAACGGACTTGTAACGAA	649bp	detection
PCV3-R	AAATGAGACACAGAGCTATATTCAG		
PCV2-P1	CCGCGGGCTGGCTGAACTT	1154bp	detection
PCV2-P2	ACCCCCGCCACCGCTACC		
PCV3-g4-F	TCCACGGAGGTCTGTAGGG	1337bp	complete sequence
PCV3-g4-F	GATTCGTTACAAGTCCGTTCTC		
PCV3-g2-F	TTGCACTTGTGTACAATTATTGCG	1075bp	complete sequence
PCV3-g2-R	ATCTTCAGGACACTCGTAGCACCAC		

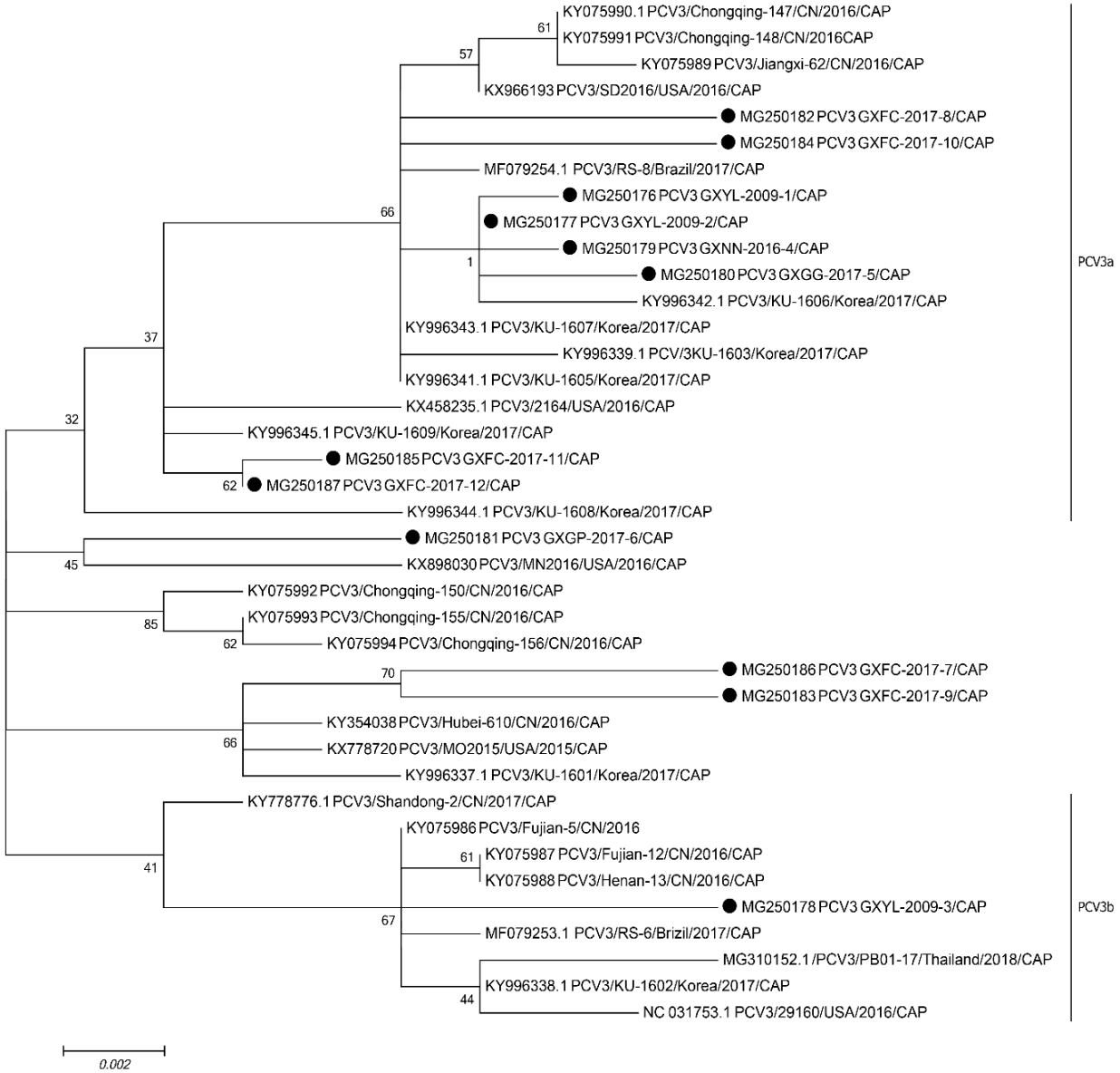

Fig. 1: Phylogenetic tree analysis based on the capsid gene (ORF2) of PCV3. Phylogenetic tree analyses using the maximum likelihood method based on the General Time Reversible model (bootstrap analysis with 1,000 replicates). Black solid circles indicate the isolates of this study.

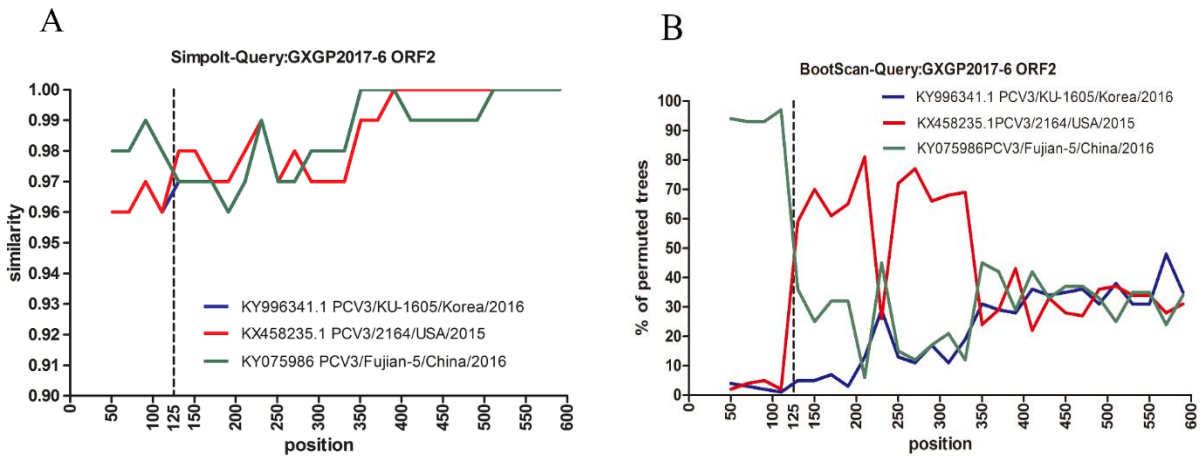

Fig. 2: Similarity plots (A) and bootscan analysis (B) on the putative recombinant sequences GXGP2017-6 from an undefined group setup as a query isolate. Different capsid genomes from PCV3a KX458235.1 PCV3/2164/USA/2015/CAP (red); KY996243. 1 PCV3/KU1606/Korea/2017/CAP (blue) and PCV3b KY075986 PCV3/Fujian-5/China /2016 (green) were setup as parental sequences. The red vertical line refers to the recombinant breakpoint. The similarity plots and bootscan analysis were constructed using the two-parameter (Kimura) distance model with a sliding window of 200 bp and step size of 20 bp, Ts/Tv=2.0. The bootscan analysis was constructed using the two-parameter (Kimura) distance model and the neighbor-joining trees model with 1000 bootstrap replicates.

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Authors contribution: QLF, CYJ, KOY and WJH designed the experiments and analyzed the data. QLF, CYJ, YQF, YFQ, QTD, GJW, YC and ZZW performed the experiments. QLF and KOY wrote the manuscript. All authors read and approved the final manuscript.

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