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Research Article

Epizootiological surveillance of porcine circoviruses in free-ranging wild boars in China

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ABSTRACT

Four species of porcine circoviruses (PCV1–4) have been reported to circulate in Chinese domestic pigs, while the epizootiology of these viruses in free-ranging wild boars in China remains unknown. In this study, tissue and serum samples collected from diseased or apparently healthy wild boars between 2018 and 2020 in 19 regions of China were tested for the prevalence of PCV1–4 infections. Positive rates of PCV1, PCV2, and PCV3 DNA in the tissue samples of Chinese wild boars were 1.6% (4/247), 58.3% (144/247), and 10.9% (27/247) respectively, with none positive for PCV4. Sequence analysis of viral genome showed that the four PCV1 strains distributed in Hunan and Inner Mongolia shared 97.5%–99.6% sequence identity with global distributed reference strains. Comparison of the *ORF2* gene sequences showed that 80 PCV2 strains widely distributed in 18 regions shared 79.5%–100% sequence identity with reference strains from domestic pigs and wild boars, and were grouped into PCV2a (7), PCV2b (31) and PCV2d (42). For PCV3, 17 sequenced strains shared 97.2%–100% nucleotide identity at the genomic level and could be divided into PCV3a (3), PCV3b (2) and PCV3c (12) based on the phylogeny of *ORF2* gene sequences. Serological data revealed antibody positive rates against PCV1 and PCV2 of 11.4% (19/167) and 53.9% (90/167) respectively. The data obtained in this study improved our understanding about the epidemiological situations of PCVs infection in free-ranging wild boars in China and will be valuable for the prevention and control of diseases caused by PCVs infection.

1. Introduction

Porcine circoviruses (PCVs) are the smallest known non-enveloped animal viruses. They contain circular single-stranded DNA genomes and belong to the genus *Circovirus* within the family *Circoviridae* (Ellis, 2014; Prinz et al., 2019). Numerous circoviruses have been found in mammals, fish, birds and insects (Opriessnig et al., 2020). In pigs, four circovirus species within the genus *Circovirus* have been identified, including the non-pathogenic porcine circovirus 1 (PCV1) (Tischer et al., 1974), the pathogenic porcine circovirus 2 (PCV2) (Allan et al., 1998), porcine circovirus 3 (PCV3) (Palinski et al., 2017) and the recently identified porcine circovirus 4 (PCV4) (Zhang et al., 2020). The viral genome sizes are similar for PCV1 (1758–1760 nt), PCV2 (1766–1769 nt), and PCV4 (1770 nt), and with a longer genome found in PCV3 (1999–2000 nt). All four PCVs show similar genomic structure with two main open reading frames (ORF1 and ORF2) oriented in opposite directions in the circular genome. ORF1, or *Rep* gene, encodes Rep protein which is involved in replication, and ORF2, or *Cap* gene, encodes the Cap protein, which is the only structural component of the virion and the dominant immunogenic antigen (Cao et al., 2018).

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PCV1 was first identified in a porcine kidney cell culture (PK-15) in 1974. It has not been associated with disease, and is genetically stable (Cao et al., 2018). PCV2 is the etiological agent of porcine circovirus-associated disease (PCVAD) and causes substantial economic losses for the pig industry worldwide (Gillespie et al., 2009; Ellis, 2014). Genotyping of the global PCV2 strains based on the full-length *ORF2* gene sequences have grouped PCV2 strains into 8 genotypes (PCV2a–h) with PCV2a, 2b and 2d as the dominant groups during different time periods (Turlewicz-Podbielska et al., 2022). PCV3 was originally identified in 2015 in the USA by metagenomic sequencing, and is associated with different porcine diseases, including cardiac and multi-systemic inflammation, porcine dermatitis and nephropathy syndrome (PDNS), reproductive failure, and the porcine respiratory disease complex and diarrhea (Phan et al., 2016; Palinski et al., 2017). PCV3 infection has been reported in many countries, including USA, China, Thailand, India, Brazil, Poland, Italy and Germany, indicating its worldwide spread (Chen et al., 2021). PCV4 was first reported in 2019 in Hunan Province, China, and has been associated with respiratory and enteric symptoms, and PDNS. It has subsequently been reported in other regions in China, including Henan, Shanxi, Inner Mongolia and Guangxi (Tian et al., 2020; Sun et al., 2021; Zhuo et al., 2021), and also in Korea (Dyk et al., 2021).

In addition to domestic pigs, PCVs have also been found to be prevalent in free-ranging wild boars in many foreign countries, including Italy (Amoroso et al., 2021; Dei Giudici et al., 2020), Korea (Song et al., 2020), Brazil (Souza et al., 2021), Austria (Auer et al., 2022), Ukraine (Rudova et al., 2022), Hungary (Cságola et al., 2006), Portugal (de Sousa Moreira et al., 2022) and Germany (Prinz et al., 2019), while the epizootiology of PCVs in Chinese free-ranging wild boars remains little research because of the difficulty of sample collection, although PCV2 infection was observed in hybrid wild boars from north-east China and PCVs in wild boar from Jiangxi Province of China (Guo et al., 2019; Wu et al., 2022). Since wild boars are considered an important reservoir of animal and zoonotic pathogens, it is important to identify the epizootiology trends of important and emerging infectious diseases in wild boars for both livestock production and public health issues (Meng et al., 2009; Li et al., 2019; Gong et al., 2022). In this study, the genetic characteristics and serological prevalence of PCVs in Chinese free-ranging wild boars were investigated.

2. Materials and methods

2.1. Sample information

Since the first outbreaks of African swine fever (ASF) in 2018 in the domestic pigs and wild boars of China (Li et al., 2019; Zheng et al., 2022), the National Forestry and Grassland Administration of China enhanced the surveillance of ASF and other important and emerging infectious diseases in wild boars, and sample collections from sick and apparently healthy wild boars in different regions were approved. Between August 2018 and November 2020, tissue samples (tonsil, spleen, liver, kidney, lung and lymph node) and 167 serum samples from 247 wild boars were collected over 19 regions in China and transported under refrigerated conditions to the laboratory and stored at −80 °C until use (Fig. 1A).

2.2. Virus detection

Tissue samples from each boar were combined and processed as 10% homogenates in minimal essential medium (MEM; Corning, USA). Clarified supernatants were then subjected to viral genomic DNA extraction using the TIANamp Genomic DNA Kit (TIANGEN, Beijing, China) according to the manufacturer's instructions. The obtained DNA served as the template for PCR detection of PCV1 (Mankertz et al., 2000), PCV2 (Kim et al., 2001), PCV3 (Ku et al., 2017) and PCV4 (Tian et al., 2020) as previously described, and the PCR amplicons were analyzed by 1% agarose gel electrophoresis.

2.3. Virus DNA sequencing

To analyze the genomic sequence of PCV1, PCV2 and PCV3, the full-length ORF genes and genomes of PCVs isolates were amplified using Taq

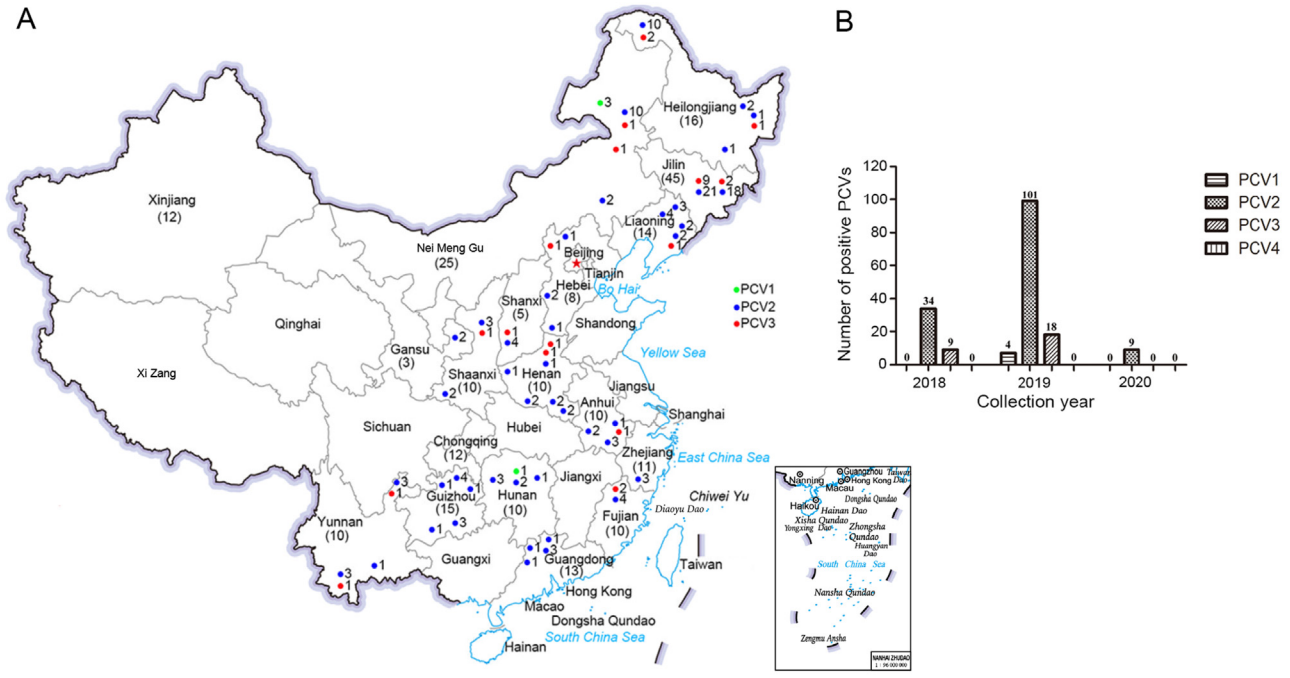

Fig. 1. Detection of PCVs infection in the tissue samples of Chinese wild boars collected between 2018 and 2020. **A** Geographical distribution of wild boars collected in this study and the positive numbers of PCVs DNA in each region. Green, blue and red circles represent PCV1, PCV2, and PCV3-positive regions respectively accompanied with the positive numbers. The number of isolates of PCVs is shown with the corresponding-colored dot. **B** The positive numbers of PCVs detected in the Chinese wild boars in different years.

DNA polymerase (TaKaRa, Dalian, China) as described previously (Cao et al., 2018; Hu et al., 2022; Xia et al., 2019; Wen et al., 2018), the PCR amplicons were separated by electrophoresis on a 1% agarose. The bands were extracted and purified using the AxyPrep DNA Gel Extraction Kit (AxyGene, USA). Then, the PCR products were ligated into the pMD-18T Vector System (TaKaRa Co. Dalian, China), and the recombinant plasmids were directly sent to Comate Bioscience (Jilin, China) for sequencing with ABI 3700. Moreover, the full-length *ORF2* gene of PCV2 isolates and the complete genomic sequences of the PCV1, PCV2 and PCV3 isolates obtained in this study have been submitted to the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) using the Sequin DNA sequence submission tool (https://www.ncbi.nlm.nih.gov/Sequin/).

2.4. Alignment and phylogenetic analysis

Phylogenetic analyses were performed with Clustal W and Molecular Evolutionary Genetics Analysis software MEGA 7.0 (Center for Evolutionary Functional Genomics, Tempe, AZ). For evolutionary analysis, phylogenetic trees based on the nucleotide sequences of *ORF2* gene was constructed using the maximum likelihood methods, with 1000 bootstrap replications and the best-fitting substitution models.

2.5. Seroprevalence of wild boars PCV1 and PCV2 infection in China

A serological survey of antibodies against PCV1 or PCV2 in wild boars was performed using the Porcine circovirus type 1 ELISA Kit (Jianglaibio, Shanghai, China) and Porcine circovirus type 2 ELISA Kit (Combetter, Hunan, China) according to the manufacturers' instructions.

3. Results

3.1. Detection of PCVs in Chinese wild boars

To simplify the procedure for PCVs detection, the tissue samples including tonsil, spleen, liver, kidney, lung and lymph node from each wild boar were mixed and homogenized, then subjected to DNA extraction and PCR detection. As showed in Fig. 1A, 4 of 247 wild boars (1.6%) from Hunan (1) and Inner Mongolia (3) were positive for PCV1, while more than half of the wild boars (58.3%, 144/247) over 17 regions were PCV2 positive, indicating the wide distribution of PCV2 in Chinese wild boars. For PCV3, 10.9% (27/247) of wild boars from 11 regions were positive, but none tested positive for PCV4. Co-infection with different PCVs was also observed, with rates of 1.6% (4/247) and 9.7% (24/247) for PCV1/PCV2 and PCV2/PCV3 respectively, but co-infection of PCV1/PCV3 was not observed. In addition, PCVs DNA in the serum samples were also detected and the positive rates were 0.6% (1/167) for PCV1, 14.4% (24/167) for PCV2, 6.6% (11/167) for PCV3, but none is positive for PCV4. Notably, all PCVs-positive serum samples were derived from wild boars carrying PCVs nucleic acids in the tissue samples. Furthermore, the prevalence of the PCVs by year is different. PCV1 was only detected in 2019, PCV3 was detected in 2018 (2.4%, 9/38) and 2019 (9.1%, 18/197), while PCV2 was detected in 2018 (89.5%, 34/38), 2019 (51.3%, 101/197), and 2020 (75.0%, 9/12) (Fig. 1B).

3.2. Phylogenetic analysis of the PCV1 isolates

The complete genome sequences of all four PCV1 strains were obtained and have been deposited in GenBank under accession numbers MZ594653–MZ594656. The four PCV1 isolates, sharing 98.5%–99.7% nt sequence identity with each other, were found to be closely related to the reference sequences from domestic pigs and wild boars (97.5%–99.6%), indicating the high genetic stability of PCV1 strains worldwide. As shown in Fig. 2, PCV1 strain CN-HuN/63 from Hunan Province clustered with the CN-HuN/2013 strain from a Chinese domestic pig and with Hun/2006 from a Hungarian wild boar, all sharing 97.6%–99.0% nt identity.

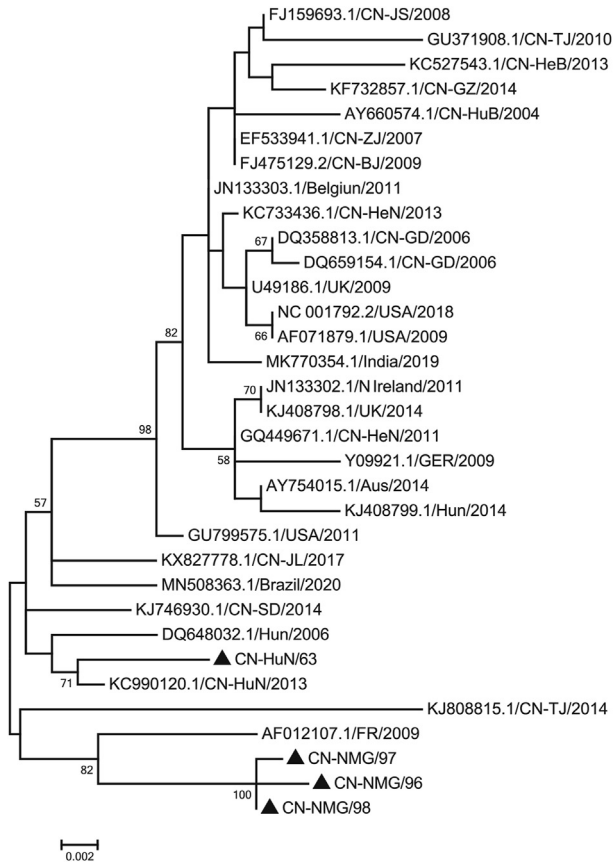

Fig. 2. Phylogenetic trees based on the nucleotide sequences of full-length *ORF2* genes of PCV1. Phylogenetic analysis was performed using MEGA v7.0 with the maximum likelihood method and 1000 bootstraps replicates, and the best fitting substitution model was Hasegawa-Kishino-Yano. PCV1 strains identified in this study are marked with black triangles (▲).

The remaining three PCV1 strains, CN-NMG/96–98, from Inner Mongolia formed a clade neighboring the single branches with strains from French (Aus/2014) and China (CN-TJ/2014) domestic pigs.

3.3. Phylogenetic analysis of the PCV2 isolates

Full-length *ORF2* gene sequences of 80 wild boar PCV2 strains were obtained and deposited in GenBank under accession numbers MZ606284–MZ606363. Sequence analysis of full-length *ORF2* genes showed that the 80 PCV2 strains shared 85.5%–100% nt and 87.6%–100% amino acid identities with each other, and 79.5%–100% nt identity with reference strains from wild boars and domestic pigs (Fig. 3A), and were grouped into PCV2a (8.8%, 7/80), PCV2b (38.8%, 31/80) and PCV2d (52.5%, 42/80), indicating that PCV2d is the dominant genotype in Chinese wild boars, similar to that circulating in Chinese domestic pigs (Zhai et al., 2014; Xia et al., 2019; Xu et al., 2021). And the prevalence of the different PCV2 genotypes by year is shown in Fig. 3B. In addition, analysis of the complete genomic sequences of 15 PCV2 strains showed genomic lengths of 1767–1768 nt (deposited in GenBank under accession numbers MZ615666–MZ615680), with the extra nucleotide in the 1768 nt genomes (CN-JN/25, CN-JN/26, CN-HLJ/53, CN-HLJ/54 and CN-Shanxi/70) being due to an insertion of T at site 1035 of viral genomes. Moreover, we identified several amino acid substitutions in the Cap proteins of PCV2a, PCV2b and PCV2d by multiple sequence alignment of deduced amino acids (Table 1). In particular, it was reported that T60 is highly conserved in previously identified PCV2a reference strains, but T60S mutation is found in the wild boar PCV2a strains CN-JL/25, CN-JL/26, CN-JL/33 and CN-HLJ/46. Similar cases also occurred in

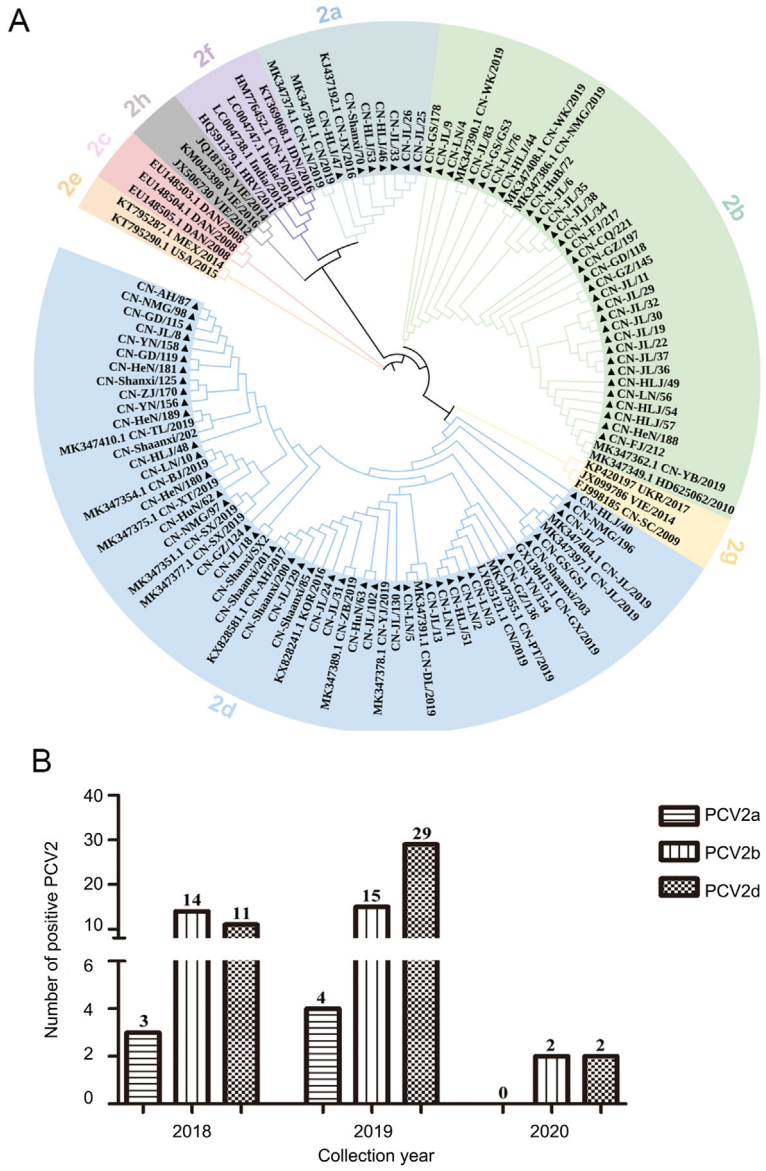

Fig. 3. Phylogenetic analysis and temporal dynamics of wild boar PCV2 strains. **A** Phylogenetic tree based on the nucleotide sequences of full-length *ORF2* genes of PCV2. Phylogenetic analysis was performed using MEGA v7.0 with the maximum likelihood method and 1000 bootstraps replicates, and the best fitting substitution model was Tamura-Nei model. PCV2 strains identified in this study are marked with black triangles (▲). **B** Temporal dynamics of different PCV2 genotype strains identified in Chinese wild boars.

wild boars PCV2b strains CN-JL/6, CN-JL/34, CN-JL/35, CN-JL/38, and CN-JL/83 (T131P), and in PCV2d strain CN-HLJ/40 (K59A and T151P) (Xia et al., 2019; Xu et al., 2021).

3.4. Phylogenetic analysis of the PCV3 isolates

Of the 27 PCV3 positive samples, the complete genome sequences of 17 were determined and have been deposited in GenBank (MZ615681–MZ615697). These sequenced strains contained two different genome sizes: 1999 nt (3) and 2000 nt (14), the shorter with a G deletion at 1149 nt. The sequenced 17 PCV3 strains shared 96.3%–99.4% nt identity with the wild boar and domestic pig reference strains. As shown in Fig. 4, PCV3 strains circulating in the Chinese free-ranging wild boars were grouped within PCV3a (3), PCV3b (2), and PCV3c (12), with each containing different amino acid residues at Cap sites 24, 27, 77, and 150 (A/V24, R/K27, S/T77 and I/L150), which are the molecular markers of the three PCV3 genotypes (Fu et al., 2018; Fux et al., 2018; Geng et al., 2019). Moreover, 12 PCV3 isolates strains of wild boars in China carry an N56D substitution at position 56 of the Cap protein (Fig. 5).

3.5. Serological survey of PCV1 and PCV2 infection

The overall seroprevalence of PCV1 and PCV2 in the examined wild boars was 11.4% (19/167) and 53.9% (90/167), respectively. PCV1 seropositive wild boars were from 10 regions: Inner Mongolia (4/23), Heilongjiang (1/9), Jilin (3/19), Hunan (1/6), Yunnan (2/10), Guangdong (2/13), Anhui (2/10), Guizhou (1/11), Zhejiang (1/11) and Henan (2/18). PCV2 seropositive samples were from 16 regions: Inner Mongolia (12/23), Liaoning (5/9), Heilongjiang (4/9), Jilin (18/19), Hunan (1/6), Shanxi (5/5), Yunnan (8/10), Xinjiang (3/6), Chongqing (2/4), Guangdong (7/13), Hebei (3/8), Shaanxi (1/1), Anhui (4/10), Guizhou (4/11), Zhejiang (3/11) and Henan (10/18) (Table 2), indicating the wide distribution of PCV2 infection in wild boars across China.

Table 1
Information about amino acid mutations in PCV2 Cap protein.

Amino acid mutation site	Genotype		
	PCV2a	PCV2b	PCV2d
8	Y/F	Y	Y/F
21	Q/S	Q	Q
47	S/A	T	T
53	F	F	I/F
57	V	I	V/I
59	A	K/R	K/A
60	T/S	T	T
63	T/S/R	R/K	R
68	A/S	A	N/A
72	L/M	M	M
76	L/I	I	I
77	D	N	N
80	V/L	L	L
86	T	S	S
88	K	P	P
89	I	R	L
90	S	S	T
91	I	V	V
121	S	S	T
123	I/V	V	V/I
130	V/F	V	V
131	M/P	T/P	T/P
133	V/S	A	A
134	P/T	T	N/T
136	Q/L	L	L
151	P	T	T/P
169	S	S	R/G/S
185	M	L	L
187	L/I	L	L
190	S	T/A	T
191	R/K	G	G
206	K	I	I
210	D	E	D/E
215	V	V	I/V
232	K	K/N	-/K/N
234	*	*	*/K
235	-	-	-/*/V

*Amino acid position encoded by the stop codon.
- No amino acid at this position.

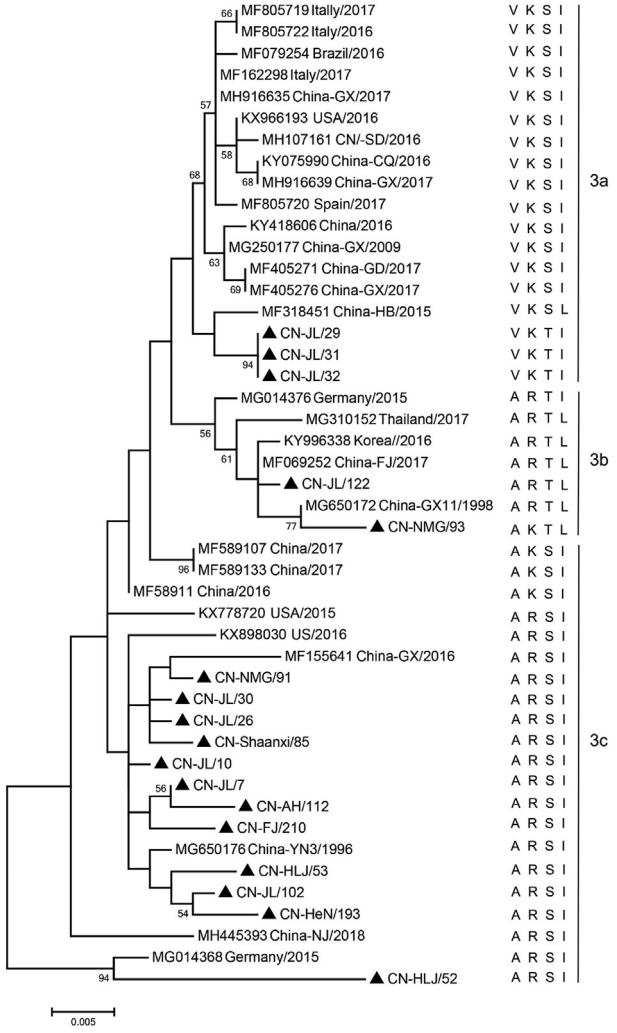

Fig. 4. Phylogenetic analysis of wild boar PCV3 strains with the reference strains based on the nucleotide sequences of full-length *ORF2* genes of PCV3. Phylogenetic analysis was performed using MEGA v7.0 with the maximum likelihood method and 1000 bootstraps replicates, and the best fitting substitution model was Hasegawa-Kishino-Yano. The genotype-specific amino acid residues in Cap proteins of PCV3 strains were attached with the phylogenetic tree. PCV3 strains identified in this study are marked with black triangles (▲).

4. Discussion

PCVs have been identified and there are currently four recognized types PCV1–4. Here, the prevalence of PCVs in Chinese wild boars is given except for Jiangxi Province (Hu et al., 2022; Wu et al., 2022). The present study shows that the wild boar populations have been infected with PCV1, PCV2 and PCV3, but not infected with PCV4. Overall, the positive rate of PCV1 DNA was 1.62% (4/247) in this study, which is lower than clinical samples of Guangxi (12.95%, 32/247) and wild boar samples of Jiangxi Province (21.7%, 30/138) (Cao et al., 2018; Hu et al., 2022), while the prevalence of PCV2 infection was found to be similar to that in the clinical samples of domestic pigs in central China (57.1%, 113/198) (Xu et al., 2021), but higher than that reported in wild boars in other parts of the world, including Italy (26.8%, 22/82) (Fanelli et al., 2022), Spain (46.8%, 89/202) (Risco et al., 2013), Hungary (20.5%, 63/307) (Cságola et al., 2006), Germany (10.7%, 6/56) (Prinz et al., 2019) and Korea (6.8%, 91/1340) (Song et al., 2020). For PCV3, 10.9% (27/247) of samples from 11 regions were positive, which is lower than that found in domestic pigs in central China (36.4%, 72/198) (Xu et al., 2021) and southern China (26.7%, 76/285) (Fu et al., 2018), and in wild boars in Germany (29.2%, 26/89) (Prinz et al., 2019), Italy (33%, 62/187) (Franzo et al., 2018) and Spain (42.7%, 221/518) (Klaumann et al., 2019). The above difference of the prevalence rates of PCVs infection between Chinese wild boars and domestic pigs or foreign wild boars may be associated with the sampling numbers and seasons, the individual health status, the etiological situations and the geographical area. Since the PCVs-positive wild boars were apparent healthy upon sample collection, it remains unknown if the positivity of PCVs infection is associated with the onset of disease in wild boars.

In this study, based on phylogenetic analysis of PCV2 *ORF2* genes, PCV2a, PCV2b and PCV2d represented 8.75% (7/80), 38.75% (31/80) and 52.5% (42/80) of the total, respectively. Samples from north-east China showed that the epizootiology of PCV2 in free-ranging wild boars was similar to that of local hybrid wild boars, in which PCV2a, PCV2b and PCV2d are prevalent (Guo et al., 2019). However, only PCV2b and PCV2d were detected in Jiangxi Province (Hu et al., 2022). In addition, only PCV2b was detected in Fujian, Hebei and Chongqing wild boars, where PCV2d and PCV2a strains were also prevalent in the local domestic pigs (Han et al., 2021; Xu et al., 2022). Recently, PCV2e, a newly identified genotype, has been reported to be prevalent in domestic pigs in southern China, including Fujian and Guangdong, while PCV2e was not detected in wild boars from these regions in this study (Xu et al., 2022). Moreover, comparison of the amino acid sequences of PCV2 Cap proteins identified amino acid mutations among different genotypes (Table 1). Some of them may be associated with the alteration of Cap protein antigenicity, for example, K59A was found to be crucial for the differential reactivity (Mahé et al., 2000; Saha et al., 2012; Xia et al., 2019b). In addition, Mahé et al. (2000) reported that 6 linear epitopes

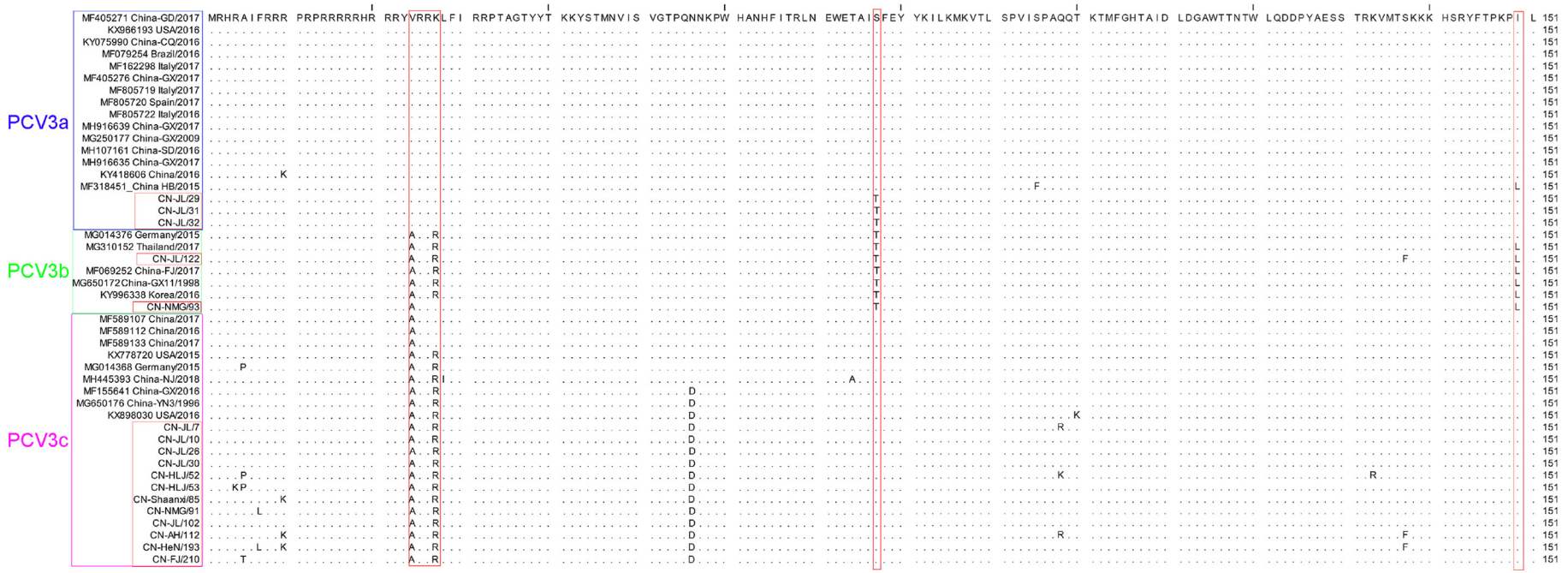

Fig. 5. Alignment of deduced amino acid sequences of PCV3 Cap proteins. Sequence comparison was performed using CLC Sequence Viewer 8 software. The major amino acid mutations are displayed in a red vertical box. Red horizontal boxes indicate the strains identified in this study.

Table 2
Prevalence of antibodies against PCV1 and PCV2 Cap protein in free-ranging wild boars from different regions of China by ELISA.

Province	No. tested	PCV1 seroprevalence		PCV2 seroprevalence	
		No. positive	Prevalence (%) (95% CI)	No. positive	Prevalence (%) (95% CI)
Jilin	19	3	15.8	18	94.7
Liaoning	9	0	0	5	55.5
Heilongjiang	9	1	11.1	4	44.4
[a]Inner Mongolia	23	4	17.4	12	52.2
Hebei	8	0	0	3	37.5
Shanxi	5	0	0	5	100
Shaanxi	1	0	0	1	100
Hunan	6	1	16.7	1	16.7
[b]Chongqing	4	0	0	2	50.0
Guangdong	13	2	15.4	7	53.8
Yunnan	10	2	20.0	8	80.0
Anhui	10	2	20.0	4	40.0
Guizhou	11	1	9.1	4	36.4
Zhejiang	11	1	9.1	3	27.3
Henan	18	2	11.1	10	55.5
[a]Xinjiang	6	0	0	3	50.0
Gansu	3	0	0	0	0
[a]Ningxia	1	0	0	0	0
Total	167	19	11.4 (6.6–16.2)	90	53.9 (46.3–61.5)

CI, confidence interval.
[a] Autonomous region.
[b] Municipality.

within the residues 25–43, 69–83, 113–127, 117–131, 169–183 and 193–207 aa of the PCV2 capsid protein were targeted by the immune system. Some of the amino acid mutations maintained among different PCV2 genotypes are located within the above epitopes, thus may alter the host immune response upon PCV2 infection. Further studies are needed to explore the function of these amino acid mutations existed between different PCV2 genotypes.

Our results confirm that PCV3 is not only present in domestic pigs, but also in apparently healthy wild boars. These sequenced strains of PCV3 contained two different genome sizes: 1999 nt (3) and 2000 nt (14), in which the shorter one possessed a G deletion at 1149 nt, in neither the ORFs nor the replication initiation sites, and was identical to a recently described strain in Guangxi (Wen et al., 2018). Moreover, more detailed investigations allowed the classification of subclades PCV3a, PCV3b and PCV3c on the basis of the molecular characteristics at sites 24, 27, 77 and 150 (Fu et al., 2018; Fux et al., 2018; Geng et al., 2019). PCV3a strains carrying A24, R/K27, S77 and I150 were dominant in the free-ranging wild boars across Jilin, Heilongjiang, Henan, Shaanxi, Anhui and Inner Mongolia. PCV3b strains carrying A24, R/K27, T77 and I/L150 were prevalent in Jilin and Guangxi. PCV3c strains carrying V24, K27, S/T77 and I/L150 were exclusively found in Jilin Province.

Additionally, the seroprevalence of PCV1 and PCV2 in wild boars sampled from different regions varied from 9.1%–20% for PCV1 and 16.7%–100% for PCV2 (Table 2). The various positive rates may be associated with the limited numbers of wild boar samples. Further study with larger sample size is needed. Moreover, 38 of 90 wild boars were positive for both PCV2 nucleic acid and antibody, and one PCV1 DNA-positive wild boar from Inner Mongolia was also positive for antibody against PCV1 Cap protein. These results indicate that persistent infections of PCV2 and PCV1 occurred among free-ranging wild boars in China. In addition, the seroprevalence of PCV2 infection in Chinese free-ranging wild boars is slightly higher than that in domestic pigs in southern China (46.0%, 819/1779) (Shuai et al., 2007) and lower than that in hybrid wild boars in northeast China (96.4%, 163/169) (Guo et al., 2019), which was also differed from that reported in the free-ranging wild boars in other countries, including Brazil (86.5%, 1129/1305) (Barbosa et al., 2016), Greece (19.1%, 18/94) (Touloudi et al., 2015) and Spain (47.9%, 314/656) (Vicente et al., 2004).

5. Conclusions

In summary, the present study firstly demonstrates the epidemiological situations of PCV1, PCV2 and PCV3 infection in free-ranging wild boars in China, and shows that PCVs circulating in Chinese wild boars are closely related to those in domestic pigs, thus emphasizing the need for constant surveillance of these PCVs, particularly PCV2, in order to avoid their transmission from wild boars to domestic pigs.

Data availability

The full-length *ORF2* gene and genome sequences of PCVs isolates obtained in this study have been deposited in the GenBank database (accession number: MZ615681–MZ615697, MZ606284–MZ606363).

Ethics statement

The sample collection from wild boars was approved by Biological Disaster Control and Prevention Center, National Forestry and Grassland Administration, China, and animal experiments were not conducted.

Author contributions

Wenjie Gong: methodology, ata curation, writing-reviewing and editing. Haiying Du: methodology, ata curation, software, data curation. Tong Wang: methodology, software, validation. Heting Sun: visualization, investigation. Peng Peng: visualization, investigation. Siyuan Qin: visualization, investigation. Haidong Geng: visualization, investigation. Zheng Zeng: visualization, investigation. Wangwang Liang: visualization, investigation. Hongquan Ling: visualization, investigation. Changchun Tu: writing-reviewing and editing. Zhongzhong Tu: methodology, ata curation, supervision, validation, writing-original draft preparation, writing-reviewing and editing.

Conflict of interest

The authors declare that they have no competing interests.

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