

## First complete genomic characterization of a porcine parvovirus 5 isolate from China

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**Abstract** To investigate porcine parvovirus 5 (PPV5) infections in swine herds in China, clinical specimens of piglet lungs were examined for the presence of PPV5 using a polymerase chain reaction method. A strain of PPV5 was detected, and its genome was sequenced and analyzed. In the sequence alignment and phylogenetic analysis, the Chinese PPV5 strain clustered into a distinct clade with the reference PPV5 strains. These results provide direct evidence that PPV5 is present in pigs in China. Extensive epidemiological studies are warranted to determine the geographic distribution of PPV5 in China.

Porcine parvovirus (PPV) is a major etiological agent of reproductive failure in sows. Gestational PPV infections are characterized by spontaneous abortion, fetal mummification, embryonic death, and subsequent infertility [17]. Panleukopenia, hepatitis, enteritis, erythrocyte aplasia, immune-complex-mediated vasculitis, and cerebellar ataxia can also be caused by PPV infections [3, 8]. In addition, PPV is an important cofactor in the development of naturally acquired porcine postweaning multisystemic wasting syndrome (PMWS) [1, 9]. PPV was first isolated from the organs of aborted fetuses in Germany and the USA in 1965 and was first reported in China in 1983 [4, 14,

15, 17]. PPV has been found in swine herds on every continent, and PPV infection is a major cause economic loss for the swine industry worldwide.

The virus family *Parvoviridae* includes two subfamilies, *Parvovirinae* and *Densovirinae*, and the subfamily *Parvovirinae* contains the five genera, *Parvovirus*, *Erythrovirus*, *Dependovirus*, *Amdovirus*, and *Bocavirus* (PBoV) [18]. PPV is a member of the family *Parvoviridae*, subfamily *Parvovirinae*, genus *Parvovirus*. Parvoviruses are small, non-enveloped, single-stranded DNA viruses with genomes that are approximately 5.0 kb in size and contain terminal palindromic sequences. There are two major open reading frames (ORFs) in parvovirus genomes, ORF1 and ORF2, which contain sequences that code for a non-structural protein and a capsid protein, respectively. Members of the genus *Bocavirus* have a third ORF in the middle of the viral genome [2].

Five different parvoviruses have been identified in pigs, including PPV1 (classic PPV), PPV2, PPV3 (porcine PARV4, hokovirus, or partetravirus), PPV4, and PBoVs, all of which are genetically divergent [5–7, 10–13, 16, 19]. Recent studies have identified novel members of the subfamily *Parvovirinae* in pig herds in the USA, which have now been classified as PPV5 strains [20, 21]. The PPV5 group is most closely related to PPV4, with overall genome sequence identities of 64.1 % to 67.3 %, when compared to PPV1. A detailed characterization of the genome of the PPV5 strains at the nucleotide and amino acid sequence levels revealed two putative ORFs that are similar to those of bovine parvovirus 2 (BPV2) but are different from those of PPV4.

To investigate PPV5 infections in swine herds in China, clinical specimens of piglet lungs were examined using a polymerase chain reaction (PCR) method, as described previously by Xiao et al. [20]. Forty-five lung tissue

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**Table 1** Sequence information for the HN01 strain and other reference PPV5 isolates

Reference virus	Accession number	Country	ORF1/ORF2 encoded protein	% similarity between HN01 and reference viruses		
				Genome sequence	NS nucleotide/protein	VP nucleotide/protein
MI216	JX896318	USA	601/991	99.0	98.7/97.8	99.3/98.9
ND564	JX896322	USA	601/991	99.1	98.8/97.7	99.3/98.9
IN273 clone 1	JX896319	USA	601/991	98.9	98.6/97.5	99.1/98.8
IA469 clone 1	JX896321	USA	601/991	98.6	98.3/97.2	98.8/98.6
IA469 clone 2	JX896320	USA	601/991	98.6	98.3/97.2	98.8/98.6
HN01	KF661535	China	599/991	/	/	/

specimens were collected during necropsy of piglets from nine farms in China. Prior to death, the animals exhibited clinical signs of PMWS, including respiratory disease, diarrhea, and systemic disease. The tissue samples were minced and homogenized in Dulbecco's modified Eagle medium (DMEM). The supernatants were collected after centrifugation, and DNA was extracted using a Tiangen Biotech DNA extraction kit (Beijing, China) according to the manufacturer's instructions.

Fragments containing PPV5 viral DNA sequences were amplified by PCR using DNA oligonucleotide primers as described previously by Xiao et al. [20]. The PCR products were cloned into the plasmid pGEM-T (Promega, Madison, WI, USA), and the viral DNA sequences were determined using automated fluorescent dye-terminator DNA sequencing and an ABI Prism 377 DNA Sequencer (Invitrogen, Carlsbad, CA, USA). The nucleotide sequences obtained in our study were submitted to GenBank under the accession number KF661535. The viral nucleotide sequences were compiled, and the amino acid sequences of the ORFs were determined using Lasergene, version 4.0, computer software (DNASTAR, Madison, WI, USA). The viral DNA sequences were aligned using ClustalW, version 1.83 ([www.clustal.org](http://www.clustal.org)). A neighbor-joining bootstrap analysis was used to construct phylogenetic trees using MEGA 5.0 (<http://www.megasoftware.net>), based on 1000 replicates.

A PPV5 strain, designated HN01, was detected in the lung tissue specimens from piglets with PMWS in Henan Province, China. The genome of this isolate was sequenced and analyzed. The organization of the HN01 genome was similar to that of other PPVs. Two distinct ORFs, ORF1 and ORF2, were identified, and the total size of the coding regions was 4895 bp. Compared with the HN01 strain, the overall genome sequence identities of the reference PPV5 strains were 98.6 % to 99.1 % (Table 1). By contrast, representative PPV4 strains had genome sequence identities of 63.9 % to 67.0 % compared with HN01 (data not shown). The nucleotide and amino acid sequence identities of ORF1 of HN01 were 98.3 % to 98.7 % and 97.2 % to

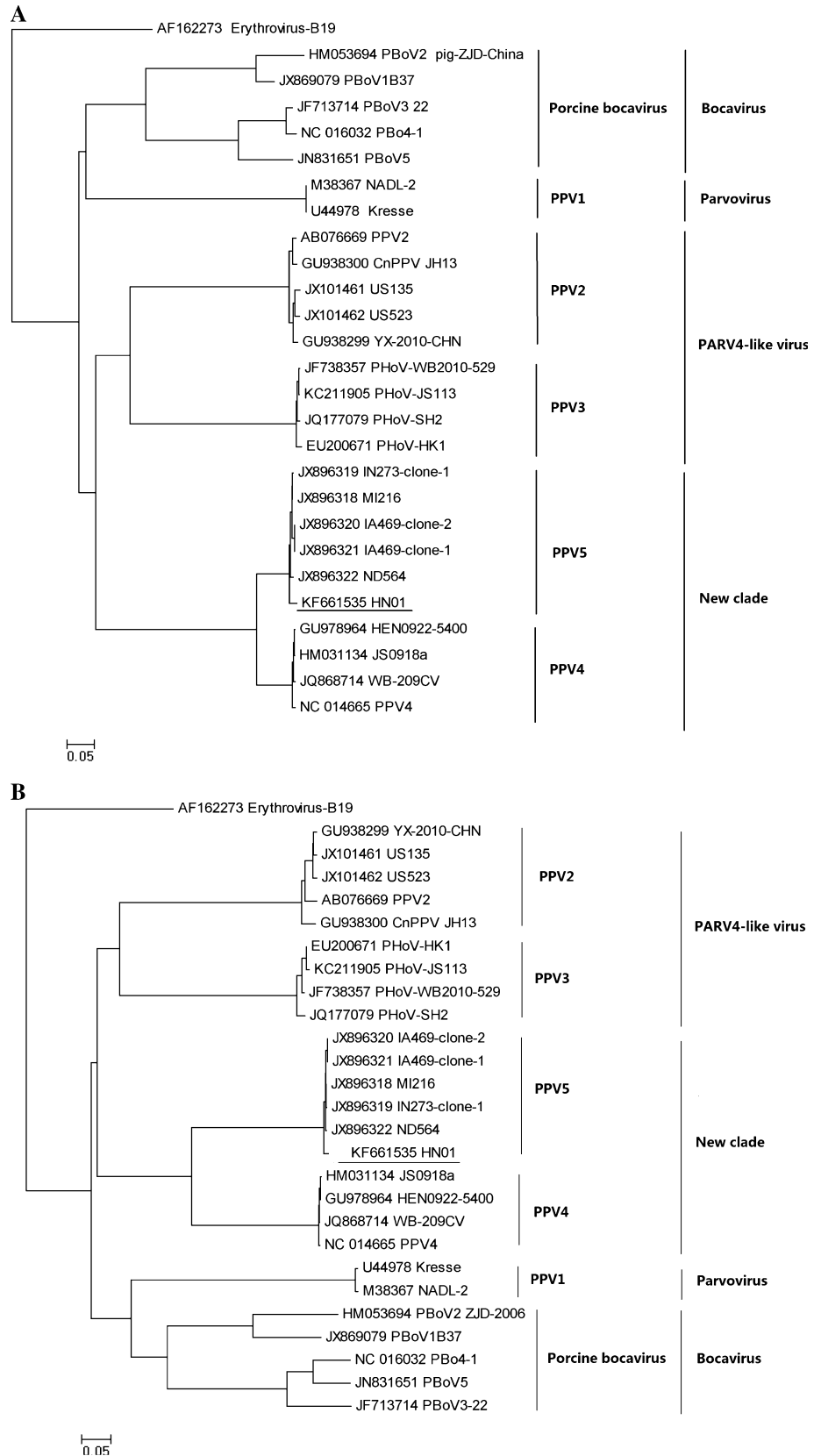
97.8 %, respectively, compared with the reference PPV5 sequences. The nucleotide and amino acid sequence identities of ORF2 of HN01 had identities of 98.8 % to 99.3 % and 98.6 % to 98.9 %, respectively, compared with the reference PPV5 sequences.

Our analysis showed that the ORF1 of the HN01 strain encodes a putative non-structural protein (NS) consisting of 599 amino acids and that ORF2 encodes a putative viral protein (VP) consisting of 991 amino acids. By contrast, the ORF1 and ORF2 of the reference PPV5 s encode an NS consisting of 601 amino acids and a VP consisting of 991 amino acids, respectively. Compared with the consensus PPV5 sequence, two amino acid deletions were identified at positions 22 and 222 in the NS of HN01. However, the effect of these deletions on HN01 NS function could not be ascertained based on the genomic data. The following nine amino acid substitutions were identified in the HN01 NS: I18L, F28Y, A57E, K139 N, N173S, A265D, P344Q, A420D, and K501E. We also identified the following 10 amino acid substitutions in the HN01 VP: K62R, P134L, K185Q, P264H, A424G, A544 V, E624 K, W696C, T780A, and N862H.

To determine evolutionary relationships between the HN01 strain and representative parvoviruses, a phylogenetic analysis was performed based on the nucleotide sequences of ORF1 and ORF2. The evolutionary trees constructed for ORF1 and ORF2 had similar topology. The results of the phylogenetic analysis indicated that the HN01 strain is most closely related to the PPV5 reference strains (Fig. 1). In addition, HN01 and other PPV5 strains formed a separate clade that was distinct from other parvoviruses, suggesting that HN01 and the other PPV5 strains could be classified in an independent genus within the subfamily *Parvovirinae*.

In conclusion, the results of our study indicate that the PPV5 strain exists in pigs in China. Sequence alignment and phylogenetic analysis demonstrated that the Chinese PPV5 strain is most closely related to other PPV5 strains, and that the Chinese PPV5 and other PPV5 strains cluster into a distinct clade. Interestingly, two unique amino acid

**Fig. 1** Phylogenetic trees constructed by the neighbor-joining method in MEGA 5.0, based on the nucleotide sequences of the nonstructural protein (ORF1, NS1) and the capsid protein (ORF2, VP1). The trees are drawn to scale, with branch lengths measured in the number of substitutions per site. The sequence determined in the present study is underlined. The sequence of erythrovirus-B19 (AF162273) is used as an outgroup to root the tree. (A) Tree constructed based on the nucleotide sequence of NS1. All positions containing gaps and missing data except ambiguous positions were included. (B) Tree constructed based on the nucleotide sequence of VP1. All positions containing gaps and missing data except ambiguous positions were included



deletions occur in the NS of the Chinese PPV5 strain, the biological significance of which remains unclear. A recent epidemiological study of pigs in the USA reported an incidence of PPV5 in lung and fecal samples of 6.6 % and 2.6 %, respectively [20]. Extensive epidemiological studies are warranted to determine the geographic distribution of the PPV5 strain in swine herds in China.

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