Genetic Analysis of Porcine Parvoviruses Detected in South Korean Wild Boars

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Research Article

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Abstract

Porcine parvovirus 1 (PPV1) is a major cause of reproductive failure in pigs; to date, six novel porcine parvoviruses (PPV2–PPV7) have been identified. Here, we detected 11 PPV1, five PPV3, three PPV4, six PPV5, five PPV6, and one PPV7 strain in Korean wild boars. PPV1, -3, and -5, and PPV6, from Korean wild boars harbor conserved motifs within the Ca^{2+} binding loop and the catalytic center of the PLA1 motif. Intra-recombination among PPV7 strains was also identified. Genetic characterization revealed that PPV1 from Korean wild boars may be similar to virulent PPV strains.

Background

Parvoviruses belonging to the family *Parvoviridae* are small, non-enveloped, single-stranded, linear DNA viruses with a genome of approximately 4.0–6.0 kb in size [1]. The PPV genome mainly encodes two open reading frames (ORFs), ORF1 and ORF2, which encode non-structural proteins (NS) and viral capsid proteins (VPs), respectively [1, 2]. Since classical porcine parvovirus (PPV1) was first identified in the 1960s, it has spread widely and become an important pathogen that causes reproductive failure in susceptible pigs [3, 4]. Various molecular methods developed during the last two decades have identified new porcine parvoviruses (PPVs) in pig herds worldwide; these are named PPV2–PPV7 [2, 5-7].

Currently, PPVs are classified taxonomically into four genera based on phylogenetic analyses of the amino acid sequences of the NS protein [1]. According to classification based upon the International Committee on Taxonomy of Viruses (ICTV), PPV1 belongs to the genus *Protoparvovirus* (species *Ungulate protoparvovirus 1*) within the subfamily *Parvovirinae*. Unlike PPV1, PPV2 and PPV3 belong to the genus *Tetraparvovirus* (species *Ungulate tetraparvovirus 3* and *Ungulate tetraparvovirus 2*, respectively), and PPV4 and PPV6 belong to the genus *Copiparvovirus* (species *Ungulate copiparvovirus 2* and *Ungulate copiparvovirus 4*, respectively) within the subfamily *Parvovirinae*. PPV5 is still not classified in the ICTV taxonomy proposal. However, PPV7 is classified as a new genus, *Chaphamaparvovirus* (*Ungulate Chaphamaparvovirus 1*) within the subfamily *Hamaparvovirinae* [8]. Recent studies report wide geographical distribution and circulation of novel PPVs in many countries, including China, Poland, Brazil, the USA, Romania, Thailand, Japan, and South Korea [6, 7, 9-15]. In Korea, sows are vaccinated with subunit VP2 vaccine and an inactivated PPV vaccine; thus the incidence of PPV in 2019 was only 15 cases, and 25 cases were recorded in 2020 (www.kahis.go.kr).

It is important to manage wild boars (*Sus scrofa L.*) because they may carry disease and play a role causing infections in domestic pigs. However, no study has examined the prevalence and genetic diversity of PPVs in wild boars in South Korea. Therefore, the aim of this study was to examine the prevalence of PPVs and to undertake genetic characterization of PPVs isolated from wild boars in Korea.

A total of 202 samples (60 lung tissue samples and 142 blood samples) were collected from wild boars captured nationwide from 2017–2018. Total viral DNA was extracted from each sample using a DNeasy Blood & Tissue kit (Qiagen Inc., Cat No. 69506; USA). The PCR methods and specific primers used to detect PPV2, PPV3, PPV4, PPV6, and PPV7 have been described previously [9, 10, 15]. The PCR conditions for primers specific for PPV1 and PPV5 (the primers were designed for this study) were as follows: initial

denaturation at 95℃ for 3 min, followed by 35 cycles of denaturation at 95℃ for 30 sec, annealing at 60℃ (PPV1) or 58℃ (PPV5) for 40 sec, extension at 68℃ for 1 min, and a final elongation step at 68℃ for 5 min. All PCR reactions were performed using the *AccuPower*® ProFi Taq PCR PreMix kit (Bioneer Inc., Korea).

Various PPVs were detected in Korean wild boars: 11 PPV1, five PPV3, three PPV4, six PPV5, five PPV6, and one PPV7. PPVs were detected in wild boars captured nationwide; the boars were of various weights (22 were 50–90 kg and nine were > 100 kg). There was also a slight difference in gender (14 males and 17 females). In this study, we detected various novel PPVs (PPV3, PPV4, PPV5, PPV6, and PPV7), as well as PPV1 (*prototype*), in lung tissues and blood samples. Although not many countries have searched for PPV in wild boars, one study reported that 44 of 842 wild boars sampled in the Transylvania region of Romania between 2006 and 2011 were positive for PPV [16]. In addition, of the 481 wild boars captured in South Korea from 2010–2011, 29% were seropositive for antibodies against PPV (142/481) [17]. Interestingly, a GenBank search suggested that only PPV1, PPV2, and PPV7 have been detected in Korean domestic pigs [17].

Two cases of co-infection with two different PPVs were identified in lung samples from wild boars, one captured in Jeonbuk (PPV3 and PPV6) and another captured in Gyeongbuk (PPV5 and PPV6). In addition, one case of co-infection with three PPVs (PPV3, PPV4, and PPV5) was identified in a wild boar captured in the Gyeongbuk region (data not shown). These results may be due to natural co-infection, which can happen frequently, and suggest the possibility of potential intra- and inter-recombination events among different types of PPVs. A previous study reported that a single PPV antigen (PPV2, -3, -4, -5, or -6) was detected in 80.2% (65/81) of DNA-positive samples identified on six commercial pig farms in Poland [6]. The remaining 16/81 PPV DNA-positive samples detected on four of the six commercial Polish pig farms were co-infected with two different PPVs, and growing pigs were more likely to be co-infected than finishing pigs [6]. The role of genome recombination within or among parvoviral species is unclear. However, recombination of rodent parvovirus was reported after a finding of phylogenetic incongruence between gene regions [18]. Another study reports evidence of natural recombination between canine parvoviruses [19].

The nucleotide sequences of the NS1/VP1 genes of PPV1 and PPV3, the REP/Cap genes of PPV4, the NS1/Cap genes of PPV5 and PPV7, and ORF1/ORF2 of PPV6 were also analyzed using specific primers, the design of which was based on the complete genomes of PPVs available in GenBank. Phylogenetic trees were reconstructed using the maximum-likelihood (ML) method and MEGA 7.0 software; the reconstruction was based on the nucleotide sequences of the NS1/VP1, REP/Cap, NS1/Cap, and ORF1/ORF2 genes [20]. ML analysis of genome (NS1/VP1, REP/Cap, NS1/Cap, and ORF1/ORF2) sequences (excluding the 5' UTR and 3' UTR regions) revealed that PPVs detected in 31 Korean wild boars belonged to six genera (PPV1, PPV3, PPV4, PPV5, PPV6, and PPV7) (Fig. 1). All PPVs from Korean wild boars belonged to one of four parvovirus genera (*Protoparvovirus*, *Tetraparvovirus*, *Copiparvovirus*, or *Chaphamaparvovirus*) (Fig. 1).

Substitution of only a few residues in the VP2 capsid protein is responsible for the distinct biological properties of the NADL2 and Kresse strains of PPV1 [21]. Only six differences (Thr-45-Ser, Ile-215-Thr, Asp-378-Gly, His-383-Gln, Ser-436-Pro, and Arg-565-Lys) were identified in the 529 amino acid sequence of the VP2 protein from the NADL2 and Kresse strains; these substitutions are responsible for the difference in

virulence between these two strains [21]. Compared with the NADL2 strain (PPV1), three PPV1 strains (17BKWB11, 18BKWB23, and 18BKWB71) detected in Korean wild boars showed the same six amino acid substitutions in the VP2 protein present in the Kresse strain (Table 1). The six amino acid substitutions in the VP2 protein of the T142 strain detected in Korean domestic pig are the same as in the Kresse strain. However, the N2 strain detected in Korean domestic pigs harbored only three amino acid substitutions in VP2 (Thr-45-Ser, Asp-378-Gly, and His-383-Gln) when compared with the NADL2 strain. D378G and H383Q, which are located on the capsid surface, abolish replication of the NADL2 strain in primary bovine testis cells; they also reduce the viral titer and cytopathic effects of Kresse in cell lines of porcine origin [22, 23]. The finding that mutations in three PPV1 from Korean wild boars are similar to those of the virulent Kresse strain may indicate high pathogenicity. Further studies based on animal models may be needed to determine whether these genetic findings are related to pathogenicity.

The conserved motifs within the Ca^{2+} binding loop (YXGXG) and the HDXXY region in the catalytic center of phospholipase A2 (PLA2) are also present in the capsid protein (VP1) of PPV1, -3, -5, and -6 detected in Korean wild boars; however, they are absent from PPV4. The Ca^{2+} binding loop (YXGXG) in PPV1, -3, -5, and -6 detected in Korean wild boars harbored conserved motifs YLGPG, YTGPG, YTGPG, and YTGPR, respectively. The PLA2 (HDXXY) motif in PPV1, -3, -5, and -6 detected in Korean wild boars also harbored conserved motifs HDEAY, HDERY, HDIRY, and YTGPR, respectively. A previous study suggests that the conserved motifs in the Ca^{2+} binding loop of PLA2 is the same as the “YXGXR” motif in PPV6, rather than the “YXGXG” or “YXGXF” motif found in most parvoviruses [24]. This PLA2 motif, which is required for parvovirus entry and infectivity, was also identified in BPV2, PPV1, PPV2, PPV3, PBoV2, and PBoV3, but not in PBoV1 and PPV4 [25–27].

The Recombination Detection Program (RDP ver. 5.5) was used to detect potential putative recombination breakpoints between PPVs isolated from Korean wild boars and the parental strains. A separate alignment of PPV1, -3, -4, -5, -6, and -7, including the full-length NS1/VP1, REP/Cap, NS1/Cap, and ORF1/ORF2 genes, was performed. The KF4 strain (PPV7) isolated from Korean domestic pigs in 2017 [28] was a recombinant between a major parent 17KWB09 (PPV7) strain in Korean wild boar and a minor parent N133 strain (PPV7), first detected in Korean domestic pigs in 2018 [28]. Potential breakpoints for the recombinant 17KWB09 strain occurred at nucleotide positions 2291 and 3494. The *P*-values for the recombinant KF4 strain were as follows: 7.001×10^{-10} for RDP; 7.060×10^{-08} for GENECONV; 6.028×10^{-10} for BootScan; 1.782×10^{-13} for MaxiChi; 5.671×10^{-10} for Chimaera; and 1.065×10^{-16} for SiScan (Fig. 2). Mosaic structure analysis of the NS1 and VP1/VP2 genes identified intra-recombination between two PPV1 strains (isolates 2074-7 and 225b) [29]. The 2074-7 strain (JX568154) was recombined from parental strains Kresse (U44978) and IDT (AY684872), whereas the 225b (AY684864) strain was recombined from parental strains 27a (AY684871) and IDT (AY684872) [29]. The intra-recombination strains identified in our study showed possible natural recombination of PPV7 between wild boars and domestic pigs. This could mean that natural recombination may be widespread due to circulation of PPV between wild boars and domestic pigs.

The probability of transmission of PPV between wild boars and domestic pigs is thought to be very high because the DNA virus survives for a long time in the field. Previous studies suggest that African swine fever virus (ASFV), a representative DNA virus, spread from wild boars to domestic pigs in Europe [30]; it is also

presumed to have spread from wild boars to domestic pigs in Korea [31]. Wild boars act as a reservoir for many viruses that cause infectious diseases in domestic pigs. A previous study suggests that strategic methods should be used to downsize the population density to prevent disease transmission to domestic pigs [17]. To prevent the spread of PPV from wild boars to domestic pigs, it is necessary to prohibit access by creating high fences around breeding pig farms.

In conclusion, we present the first report of novel PPVs detected in Korean wild boars. We detected six of the seven PPVs (PPV1, -3, -4, -5, -6, and -7, but not PPV2). Intra-recombination among PPV7 strains was also identified. Korean wild boar PPV1 strains may show pathogenicity similar to that of the Kresse strain because they harbor the same six amino acid substitutions in the VP2 protein.

Declarations

Acknowledgments

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Data availability statement

The gene sequences of 31PPV strains (Accession number: MT846928-MT846941, MT877649-MT877650, and MW711828-MW711842) detected from Korean wild boars have been deposited in GenBank.

Conflict of interest

The authors declare no conflicts of interest.

Ethical statement

All animal experiments were approved by the Animal and Plant Quarantine Agency of the Ministry of Agriculture Food and Rural Affairs.

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Table

Table 1.

Substitutions in the VP2 amino acid sequence of strains detected in Korean wild boars (compared with the NADL2 strain)

PPV strain	Position in the VP2 amino acid sequence of PPV1 (*Protoparvovirus*)												
	45	53	56	144	164	215	320	378	383	407	414	436	565
NADL2[a]	Thr	Gln	Gly	Glu	Ile	Ile	Ile	Asp	His	Lys	Ala	Ser	Arg
Kresse[b]	Ser	-	-	-	-	Thr	-	Gly	Gln	-	-	Pro	Lys
17KWB07[c]	Ser	-	-	-	-	Thr	Thr	Gly	Gln	-	-	Pro	Lys
17KWB26[c]	Ser	-	-	Ala	-	Thr	-	Gly	Gln	-	-	Pro	Lys
17KWB27[c]	Ser	-	Val	-	-	Thr	-	Gly	Gln	-	-	Pro	Lys
17KWB28[c]	Ser	His	-	-	Val	Thr	-	Gly	Gln	-	-	Pro	Lys
17KWB38[c]	Ser	-	-	-	-	Thr	-	Gly	Gln	Arg	Thr	Thr	Lys
17BKWB11[c]	Ser	-	-	-	-	Thr	-	Gly	Gln	-	-	Pro	Lys
18BKWB23[c]	Ser	-	-	-	-	Thr	-	Gly	Gln	-	-	Pro	Lys
18BKWB29[c]	Ser	-	-	-	-	Thr	Thr	Gly	Gln	-	-	Ala	Lys
18BKWB71[c]	Ser	-	-	-	-	Thr	-	Gly	Gln	-	-	Pro	Lys
18KWB04[c]	Ser	-	-	-	-	Thr	Thr	Gly	Gln	-	-	Pro	Lys
18KWB13[c]	Ser	-	-	-	-	Thr	-	Gly	Gln	-	-	Thr	Lys

[a]Reference vaccine strain = NADL2 strain (PPV1; *Protoparvovirus*).

[b]Reference virulent strain = Kresse strain (PPV1).

[c]Eleven strains detected from Korean wild boars (PPV1).

-: No substitutions in the VP2 amino acid sequence.

Figures

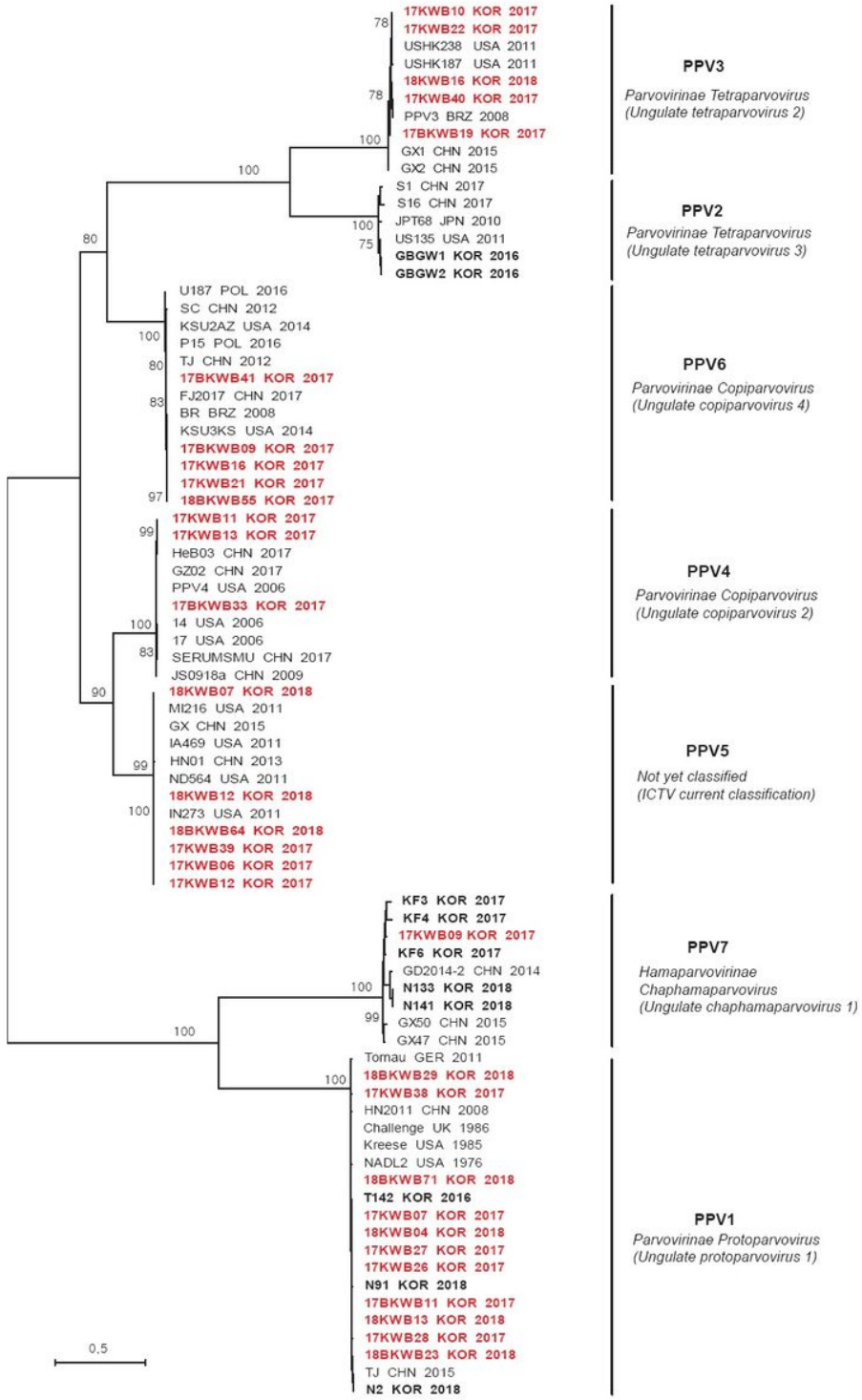

Figure 1

Maximum-likelihood tree analysis based on combining the non-structural and structural gene sequences [NS1/VP1 (PPV1 and 3), REP/Cap (PPV4), NS1/Cap (PPV5 and PPV7), and ORF1/ORF2 (PPV6) sequences]. The maximum-likelihood method (Tamura-Nei model, Gamma distributed with Invariant sites) was constructed using 1,000 bootstrap analyses in the MEGA 7.0 program. The NS1/VP1 sequences of 31 PPVs from Korean wild boars were compared with 50 reference sequences (including seven PPVs) from several countries. Korean wild boar PPVs and Korean domestic pig PPVs are denoted by red bold and black bold

letters, respectively. The notation is presented in the following order: country and year of isolation. The national abbreviations are as follows: KOR (Korea), USA (United States of America), BRZ (Brazil), CHN (China), JPN (Japan), POL (Poland), GER (Germany), and UK (the United Kingdom). The Log likelihood (Log L) for genome sequences is -28543.99. Only bootstrap values ≥70 are indicated on the nodes. The scale bar indicates the number of nucleotide substitutions per site.

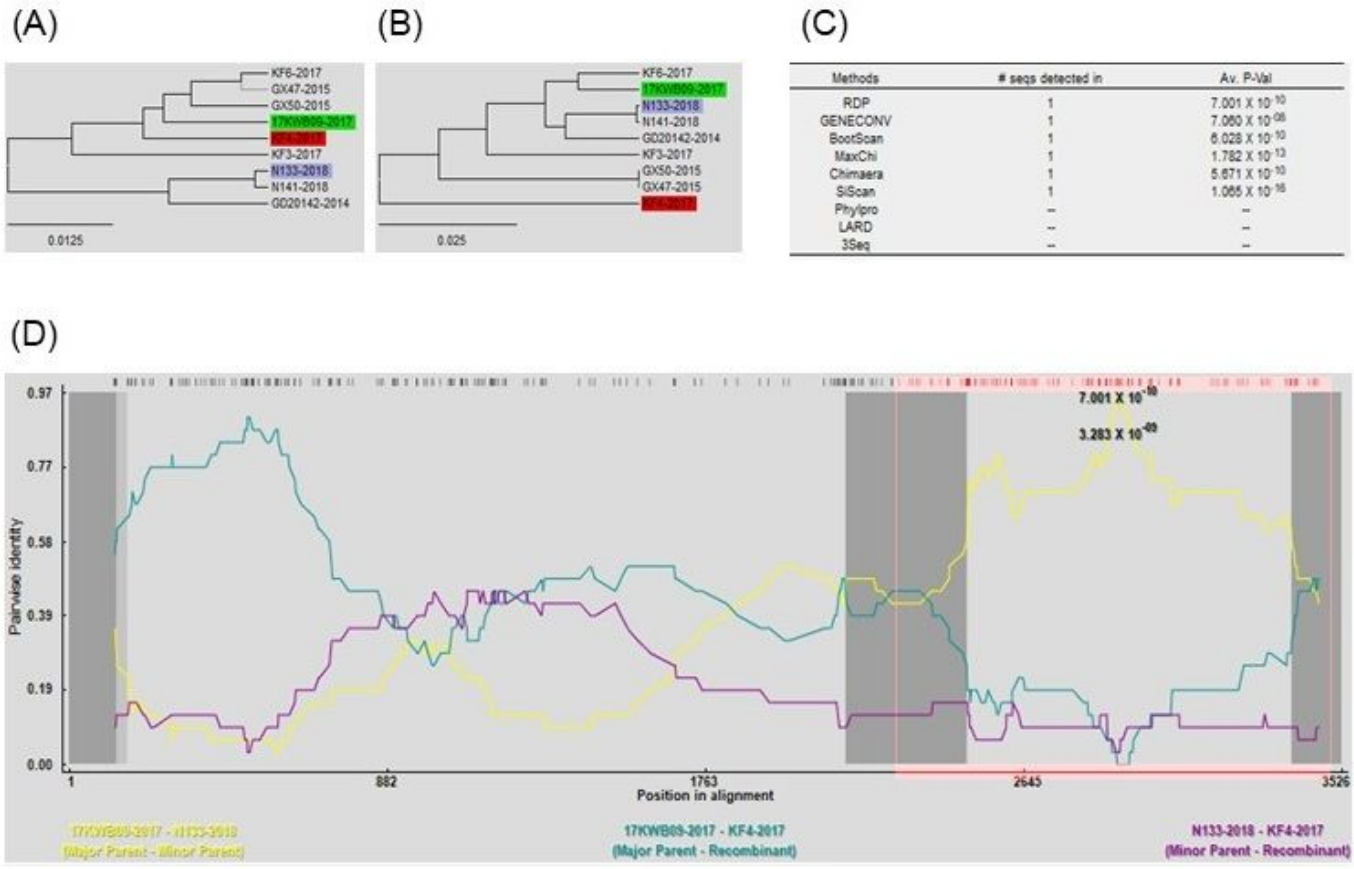

Figure 2

Recombination events detected in the NS1/Cap of PPV7 using six algorithms implemented in the RDP5.5 program. The UPGMA of regions derived from major parents (1–2290 and 3495–3526) (A) and the UPGMA of regions derived from minor parents (2291–3494) (B). Colored letters indicate potential recombinant (red), potential major parent (green), and potential minor parent (purple). The average P-value was measured by the six algorithms (C). The beginning and end breakpoints (99% confidence interval) calculated from the RDP graph were 2150–2490 (gray) and 3387–161 (gray), respectively (D). Recombination events were analyzed using different algorithms and default settings (a Bonferroni corrected and highest acceptable P-value cut-off of 0.01).