Brief Report

Genetic analysis of porcine parvoviruses detected in South Korean wild boars

Gyu-Nam Park1 · Sok Song1 · Ra Mi Cha1 · SeEun Choe1 · Jihye Shin1 · Song-Yi Kim1 · Bang-Hun Hyun1 · Bong-Kyun Park1,2 · Dong-Jun An1

Received: 5 December 2020 / Accepted: 30 March 2021 / Published online: 17 May 2021
© The Author(s), under exclusive licence to Springer-Verlag GmbH Austria, part of Springer Nature 2021

Abstract

Porcine parvovirus 1 (PPV1) is a major cause of reproductive failure in pigs. To date, six additional porcine parvoviruses (PPV2–PPV7) have been identified. In this study, we detected 11 PPV1 strains, five PPV3 strains, three PPV4 strains, six PPV5 strains, five PPV6 strains, and one PPV7 strain in Korean wild boars. PPV1, -3, and -5, and PPV6 from Korean wild boars harbor conserved motifs within the Ca²⁺ binding loop and the catalytic center of the PLA1 motif. Intra-species recombination among PPV7 strains was also identified. Genetic characterization revealed that PPV1 from Korean wild boars may be similar to virulent PPV strains.

Parvoviruses, members of the family Paroviridae, 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 contains two major 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 allowed the identification of 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 analysis of the amino acid sequence of the NS protein [1]. According to the International Committee on Taxonomy of Viruses (ICTV), PPV1 belongs to the genus Protoparvovirus (species Ungulate protoparvovirus 1) within the subfamily Parovirinae. 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 Parovirinae. PPV5 is still not classified in the ICTV taxonomy proposal. However, PPV7 is classified as a member of a new genus, Chaphamaparvovirus (species Ungulate Chaphamaparvovirus 1) within the subfamily Hamaparvovirinae [8]. Recent studies have found a 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 a VP2 subunit vaccine and an inactivated PPV vaccine, and 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 to 2018. Total viral DNA was extracted from each sample using a DNeasy Blood & Tissue...
kit (QIAGEN Inc., USA, cat. no. 69506). 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°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 60°C (PPV1) or 58°C (PPV5) for 40 s, extension at 68°C for 1 min, and a final elongation step at 68°C for 5 min. All PCR reactions were performed using an Accupower® ProFi Taq PCR Pre-Mix 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 were 17 females and 14 males. 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 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 coinfection 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 coinfection 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 coinfection, which can happen frequently, and suggest the possibility of potential intra- and inter-species 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 PPV DNA-positive samples detected on four of the six commercial Polish pig farms were coinfected with two different PPVs, and growing pigs were more likely to be coinfected 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 found 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 genome sequences of PPVs available in the GenBank database. Phylogenetic trees were constructed using the maximum-likelihood (ML) method in MEGA 7.0 software. The trees were 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 the PPVs detected in 31 Korean wild boars belonged to six types (PPV1, PPV3, PPV4, PPV5, PPV6, and PPV7) (Fig. 1), and all 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 sequences of the VP2 proteins of 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 that are present in the Kresse strain (Table 1). The T142 strain, detected in a Korean domestic pig, also has the same six amino acid substitutions as in the Kresse strain. However, the N2 strain, detected in Korean domestic pigs, had 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 isolates from Korean wild boars are similar to those of the virulent Kresse strain might 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 had the sequences YLGPG, YTGPG, and YTGPR, respectively. The PLA2 (HDXXY) motif in PPV1, -3, -5, and -6 detected in Korean wild boars had the sequence HDEAY, HDERY, HDIRY, and YTGPY, respectively. A previous study suggested that the conserved motifs in the Ca$^{2+}$ binding loop of PLA2 are the same as the “YXGXG” motif in PPV6, rather than the “YXGXG” or “YXGXF” motif found in most parvoviruses.
Porcine parvoviruses detected in South Korean wild boars

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,
The KF4 strain (PPV7), isolated from Korean domestic pigs in 2017 [28], is a recombinant between the major parent 17KWB09 (PPV7) from Korean wild boar and the minor parent N133 (PPV7), which was 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: \( 7.001 \times 10^{-10} \) for RDP; \( 7.060 \times 10^{-08} \) for GENECONV; \( 6.028 \times 10^{-10} \) for BootScan; \( 1.782 \times 10^{-13} \) for MaxiChi; \( 5.671 \times 10^{-10} \) for Chimaera; and \( 1.065 \times 10^{-16} \) for SiScan (Fig. 2). Mosaic structure analysis of the NS1 and VP1/VP2 genes identified intra-species recombination between two PPV1 strains (isolates 2074-7 and 225b) [29]. The 2074-7 strain (JX568154) is a recombinant of the parental strains Kresse (U44978) and IDT (AY684872), whereas the 225b (AY684864) strain is a recombinant of the parental strains 27a (AY684871) and IDT (AY684872) [29]. The identification of intra-species recombinants in our study suggests that natural recombination of PPV7 strains from wild boars and domestic pigs might have occurred, and 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 have suggested 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. It has been recommended that a strategy be employed 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 prevent access by building 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 PPV types (PPV1, -3, -4, -5, -6, and -7, but not PPV2). Intra-species recombination between 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.

### Table 1 Substitutions in the VP2 amino acid sequence of strains detected in Korean wild boars in comparison to the NADL2 strain

| PPV strain | Position in the VP2 amino acid sequence of PPV1 (Protoparvovirus) |
|------------|---------------------------------------------------------------|
|            | Position (Number) 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 - Pro Lys |
| 18BKWB71\(^c\) | Ser - - - - Thr - Gly Gln - Pro Lys |
| 18BKWB04\(^c\) | Ser - - - - Thr Thr Gly Gln - Pro Lys |
| 18BKWB13\(^c\) | Ser - - - - Thr - Gly Gln - Thr Lys |

\(^a\)Reference vaccine strain NADL2 (PPV1; genus Protoparvovirus)
\(^b\)Reference virulent strain Kresse (PPV1)
\(^c\)Eleven strains detected from Korean wild boars (PPV1)

-, no substitutions in the VP2 amino acid sequence
Porcine parvoviruses detected in South Korean wild boars

Acknowledgements
This project was supported by grants (project code no. B-1543083-2019-21-02) from the Animal and Plant Quarantine Agency, Republic of Korea.

Data availability
The gene sequences of 31PPV strains (accession numbers MT846928-MT846941, MT877649-MT877650, and MW711828-MW711842) detected in Korean wild boars have been deposited in the GenBank database.

Declarations

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.

Fig. 2 Recombination events detected in the NS1/Cap of PPV7 using six algorithms implemented in the RDP5.5 program. (A) The unweighted pair group method with arithmetic mean (UPGMA) of regions derived from major parents (1–2290 and 3495–3526). (B) The UPGMA of regions derived from minor parents (2291–3494). Colored letters indicate potential recombinant (red), potential major parent (green), and potential minor parent (purple). (C) Average P-value measured by the six algorithms. 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 analyzed using different algorithms with default settings (a Bonferroni-corrected and highest acceptable P-value cutoff of 0.01).

References

1. Streck AF, Canal CW, Truyen U (2015) Molecular epidemiology and evolution of porcine parvoviruses. Infect Genet Evol 36:300–306
2. Streck AF, Truyen U (2019) Porcine parvovirus. Curr Issues Mol Biol 37:33–46
3. Cartwright S, Huck R (1967) Viruses isolated in association with herd infertility abortions and stillbirths in pigs. Vet Rec 81:196–197
4. Johnson R, Collings D (1969) Experimental infection of piglets and pregnant gilts with a parvovirus. Vet Rec 85:446–447
5. Cadar D, Csagola A, Kiss T, Tuboly T (2013) Capsid protein evolution and comparative phylogeny of novel porcine parvoviruses. Mol Phylogenet Evol 66(1):243–253
6. Cui J, Biernacka K, Fan J, Gerber PF, Stadejek T, Opriessnig T (2017) Circulation of porcine parvovirus types 1 through 6 in serum samples obtained from six commercial polish pig farms. Transbound Emerg Dis 64:1945–1952
7. Milek D, Wozniak A, Guzowska M, Stadejek T (2019) Detection patterns of porcine parvovirus (PPV) and novel porcine parvoviruses 2 through 6 (PPV2-PPV6) in Polish swine farms. Viruses 11(5):474
8. Pénzes JJ, Söderlund-Venermo M, Canuti M, Eis-Hübinger AM, Hughes J, Cotmore SF, Harrach B (2020) Reorganizing the family Parvoviridae: a revised taxonomy independent of the canonical approach based on host association. Arch Virol 165(9):2133–2146

9. Cságola A, Lorincz M, Cadar D, Tombácz K, Biksi I, Tuboly T (2012) Detection, prevalence and analysis of emerging porcine parvovirus infections. Arch Virol 157:1003–1010

10. Ni J, Qiao C, Han X, Han T, Kang W, Zi Z, Cao Z, Zhai X, Cai X (2014) Identification and genomic characterization of a novel porcine parvovirus (PPV6) in China. Virol J 11:203

11. Ouh IO, Park S, Lee JY, Song JY, Cho IS, Kim HR, Park CK (2018) First detection and genetic characterization of porcine parvovirus 7 from Korean domestic pig farms. J Vet Sci 19:855–857

12. Saechow P, Mawatari T, Ikeda H (2014) Coexistence of multiple strains of porcine parvovirus 2 in pig farms. Microbiol Immunol 58(7):382–387

13. Sun J, Huang L, Wei Y, Wang Y, Chen D, Du W, Wu H, Liu C (2015) Prevalence of emerging porcine parvoviruses and their co-infections with porcine circovirus type 2 in China. Arch Virol 160:1339–1344

14. Xiao CT, Gimenez-Lirola LG, Jiang YH, Halbur PG, Opriessnig T (2013) Characterization of a novel porcine parvovirus tentatively designated PPV5. PLoS ONE 8(6):e65312

15. Xing X, Zhou H, Tong L, Chen Y, Sun Y, Wang H, Zhang G (2018) First identification of porcine parvovirus 7 in China. Arch Virol 163(1):209–213

16. Cadar D, Dan A, Tombacé K, Lorincz M, Kiss T, Becskei Z, Spino M, Tuboly T, Csgola A (2012) Phylogeny and evolutionary genetics of porcine parvovirus in wild boars. Infect Genet Evol 12(6):1163–1171

17. Jeoung HY, Lim SI, Kim JJ, Cho YY, Kim YK, Song JY, Hyun BH, An DJ (2015) Serological prevalence of viral agents that induce reproductive failure in South Korean wild boar. BMC Vet Res 11:78

18. Lukashov VV, Goudsmit J (2001) Evolutionary relationships among paroviruses: virus-host coevolution among autonomous primate paroviruses and links between adeno-associated and avian paroviruses. J Virol 75:2729–2740

19. Mochizuki M, Ohshima T, Uye Y, Yachi A (2008) Recombination between vaccine and field strains of canine parvovirus is revealed by isolation of virus in canine and feline cell cultures. J Vet Med Sci 70:1305–1314

20. Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874

21. Zimmermann P, Ritzmann M, Selbitz HJ, Heinritzi K, Truyen U (2006) VP1 sequences of German porcine parvovirus isolates define two lineages. J Gen Virol 87:295–301

22. Bergeron J, Hebert B, Tijssen P (1996) Genome organization of the Kresse strain of porcine parvovirus: identification of the allo-tropic determinant and comparison with those of NADL-2 and field isolates. J Virol 70:2508–2515

23. Fernandez S, Boisvert M, Tijssen P (2011) Genetic elements in the VP region of porcine parvovirus are critical to replication efficiency in cell culture. J Virol 85:3025–3029

24. Cheng WX, Li JS, Huang CP, Yao DP, Liu N, Cui SX, Jin Y, Duan ZJ (2010) Identification and nearly full-length genome characterization of novel porcine bocaviruses. PLoS ONE 5:e13583

25. Za’dori Z, Szélei J, Lacoste MC, Li Y, Gariepy S, Raymond P, Allaire M, Nabi IR, Tijssen P (2001) A viral phospholipase A2 is required for parvovirus infectivity. Dev Cell 1:291–302

26. Giord A, Wobus CE, Zadori Z, Ried M, Leite K, Tijssen P, Klein-schmidt JA, Halle M (2002) The VP1 capsid protein of adeno-associated virus type 2 is carrying a phospholipase A2 domain required for virus infectivity. J Gen Virol 83:973–978

27. Dorsch S, Liebsch G, Kaufmann B, von Landenberg P, Hoffmann JH, Drobnik W, Modrow S (2002) The VP1 unique region of porcine parvovirus B19 and its constituent phospholipase A2-like activity. J Virol 76:2014–2018

28. Ouh IO, Park S, Lee JY, Song JY, Cho IS, Kim HR, Park CK (2018) First detection and genetic characterization of porcine parvovirus 7 from Korean domestic pig farms. J Vet Sci 19(6):855–857

29. Leal E’, Villanova FE, Lin W, Hu F, Liu Q, Liu Y, Cui S (2012) Interclade recombination in porcine parvovirus Strains. J Gen Virol 93:2692–2704

30. Guinat C, Gogin A, Blome S, Keil G, Pollin R, Pfeiffer DU, Dixon L (2016) Transmission routes of African swine fever virus to domestic pigs: current knowledge and future research directions. Vet Rec 178(11):262–267

31. Kim HJ, Cho KH, Ryu JH, Jang MK, Chae HG, Choi JD, Nah JJ, Kim YJ, Kang HE (2020) Isolation and genetic characterization of African swine fever virus from domestic pig farms in South Korea, 2019. Viruses 12:1237

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.