KJVR
Korean Journal of Veterinary Research

Short Communication

Virology

pISSN 2466-1384 · eISSN 2466-1392
Korean J Vet Res 2024;64(1):e4
https://doi.org/10.14405/kjvr.20230036

*Corresponding author:
**Young Soo Lyoo**
College of Veterinary Medicine, Konkuk University, 120 Neungdong-ro, Gwangjin-gu, Seoul 05029, Korea
Tel: +82-2-450-3719
E-mail: lyoo@konkuk.ac.kr
https://orcid.org/0000-0002-0048-1911

Conflict of interest:
The authors declare no conflict of interest.

**Received:** Sep 5, 2023
**Revised:** Feb 20, 2024
**Accepted:** Feb 23, 2024





# Notable mutations of porcine parvovirus 1 and 4 circulating in commercial pig farms in South Korea

Beomsu Park[1], Jihyeon Hong[1], Jongsu Jun[1], An Kook Choi[1], Choi Kyu Park[2], Young Soo Lyoo[1,*]

[1]College of Veterinary Medicine, Konkuk University, Seoul 05029, Korea
[2]College of Veterinary Medicine, Kyungpook National University, Daegu 41566, Korea

## Abstract

In this study, almost complete genomic sequences of porcine parvovirus (PPV)1 and PPV4 circulating in commercial pig farms in South Korea were obtained and analyzed. Important mutations that may be precursors to host changes, such as premature stop codons of PPV1 and frameshift mutations of PPV4, were observed in these sequences. A 27a-like strain of PPV1, known to show a lack of cross- neutralization against existing commercial vaccine strains, was identified by phylogenetic analysis. Given the active genetic evolution, the additional precursors to host changes and emerging new genotypes of PPVs need to be monitored through continuous sampling and genetic analysis.

**Keywords:** porcine parvovirus; premature stop codon; frameshift; 27a-like strain

*Parvoviridae* is a family of animal viruses that infect and cause various diseases in many mammals, including pigs, dogs, cats, and humans [1]. Parvovirus is a single-stranded DNA virus, approximately 5 kb in genomic length. A complex palindromic hairpin structure of approximately 120 to 200 bases is necessary for DNA replication in both terminal sequences of the virus [2].

The most widely known porcine parvovirus is porcine parvovirus (PPV)1, a major cause of reproductive failure syndrome in pigs. This reproductive failure syndrome is referred to as SMEDI and includes stillbirths, mummified fetuses, early embryonic death, and infertility [3]. Recently, a new dominant strain of PPV1 called the 27a-like strain has emerged in Europe, which shows a lack of neutralization against the existing vaccine strains [4]. Identifying whether the 27a-like strain exists in Korean pig populations is necessary. PPV4 was first discovered in the United States in 2005 and belongs to copiparvovirus, such as Bovine parvovirus 2 [5]. Unlike other parvoviruses, it has Bocaviruses like ORF3. PPV4 infections may persist in pigs, but its pathogenicity has not been revealed.

The transfer of viruses between different hosts to produce a new self-sustaining epidemic is rare. A rare example is the canine parvovirus, which adapted to dogs as the host range changes to the feline parvovirus [6]. The canine adaptation of the parvoviruses involved capsid protein changes, altering the recognition of the host transferrin receptors, allowing canine transferrin receptor binding, and its use as a receptor for cell infection [7]. Hence, the parvovirus must be monitored for these changes because its host may change due to protein mutations. As some viruses have spread from pigs to other species, including humans [6,8], genetic alterations in PPVs should be monitored, and signs of changes in host specificity should be

closely scrutinized. From 2016 to 2022, 120 oral fluid samples for each breeding stage were submitted for laboratory diagnosis from domestic pig farms in South Korea.

Viral DNA was extracted from the oral fluid and identified by polymerase chain reaction (PCR) using a known multiplex primer set [9]. The multiplex PCR conditions were as follows: pre-denaturation at 95°C for 10 minutes; 35 cycles of denaturation at 95°C for 30 seconds, varied annealing temperature for 30 seconds, and extension at 72°C for 1 minute; and a final extension at 72°C for 5 minutes. The PCR products were evaluated by 2% agarose gel electrophoresis. Positive samples were sequenced using sequencing primers. The new primer was designed using the Primer3 tool of the Geneious Prime v2021.2.2 (Biomatters, New Zealand) according to the sequence data obtained from each sample. The amplified DNA was run on a 2% agarose gel, and the appropriate DNA band was extracted using a QIAquick Gel Extraction kit (QIAGEN, USA) and subjected to sequence analysis (Bionics, Korea). Table 1 lists the oligonucleotide sequence of each primer set used in this study. The obtained sequences were evaluated and assembled with Geneious Prime v2021.2.2. The complete nucleotide sequences obtained were compared with the sequence data available in GenBank, and sequence alignment was carried out using the MUSCLE included in Geneious Prime [10]. Translation of the nucleotide sequences into amino acid sequences and the alignment of the amino acid sequences were also carried out using the MUSCLE included in Geneious Prime. Phylogenetic analysis was performed using the PHYML in Geneious prime software in Hasegawa Kishino Yano (HKY) method mode with 500 bootstrap replicates [11]. The maximum likelihood tree was analyzed based on VP1 of PPV1 to determine the presence of a strain genetically and antigenically different from the existing vaccine strain, such as the 27a-like strain [2,12]. The VP1 sequence of canine parvoviruses (M19296.1) was used as an outgroup.

**Table 1.** Sets of primers used to diagnose and obtain almost complete genome sequences

| Primers | Sequence (5′–3′) | Nucleotide position of primers for target | Accession number | Product length (bp) |
|---|---|---|---|---|
| PPV1_mF | AGTTAGAATAGGATGCGAGGAA | 1761–1781 | NC_001718 | 163 |
| PPV1_mR | TGCTTGGTAACCTTTCTTTACC | 1902–1923 | | 163 |
| PPV1_F1 | CACTTCGCTCCAGAGACACAGCTA | 244–267 | NC_001718 | 1,006 |
| PPV1_R1 | TTTCTCCTCCGGTTTGAGCC | 1230–1249 | | 1,006 |
| PPV1_F2 | ACAACGGTTACTACAGCTCAGG | 1075–1096 | | 1,011 |
| PPV1_R2 | CTGAATCTGGCGGTGTTGGA | 2166–2185 | | 1,011 |
| PPV1_F3 | CAACAACTACGCAGCAACTCC | 2061–2081 | | 1,028 |
| PPV1_R3 | CGCCACCCCTGATTCTGAA | 3070–3088 | | 1,028 |
| PPV1_F4 | CCATACACACCAGCAGCACC | 3359–3378 | | 993 |
| PPV1_R4 | AGGTTGTTGAGGAGAGTCAGC | 4331–4351 | | 993 |
| PPV1_F5 | GCACTAAACAATACTGCACCTGT | 4163–4185 | | 883 |
| PPV1_R5 | TGTCTTTAGGGTGGGTGGGT | 5026–5045 | | 883 |
| PPV1_F6 | GGGGGAGGGCTTGGTTAGAATCAC | 2974–2997 | | 840 |
| PPV1_R6 | ACCTGAGCTGGCCTAATTGC | 3794–3813 | | 840 |
| PPV1_F7 | ATCAACAGGCACCACTAAACCT | 4041–4062 | | 814 |
| PPV1_R7 | ATAGTAAACACATGAGAGCTTGTT | 4831–4854 | NC_001718 | 814 |
| PPV4_mF | CTGAGACTGAATTCATCCCTG | 2609–2629 | MW073110 | 592 |
| PPV4_mR | ATCAGAATCATGTATGGTCTGC | 3179–3200 | | 592 |
| PPV4_F1 | GTCACTTCCGGGTCAAAGGT | 311–330 | MW073110 | 1,245 |
| PPV4_R1 | CAAGCATTTTCTCCCCGGTG | 1536–1555 | | 1,245 |
| PPV4_F2 | ATAAACACGGGGCCTGGAAG | 1322–1341 | | 1,387 |
| PPV4_R2 | AGGGGCACTTATCAACTACTGC | 2687–2708 | | 1,387 |
| PPV4_F3 | ATATCGTGGGGGCTCAGGTA | 2018–2037 | | 1,087 |
| PPV4_R3 | TTGTGAGGCATCCATGGGTC | 3085–3104 | | 1,087 |
| PPV4_F4 | CTGCTGAGACGGTGGAGATT | 2585–2604 | | 1,385 |
| PPV4_R4 | GGAGGAGATGCACGTTTGGA | 3950–3969 | | 1,385 |
| PPV4_F5 | GGTCATCGCTACACAGGTCC | 3575–3594 | | 977 |
| PPV4_R5 | TGTCCCGGTAGATGTCCTGG | 4532–4551 | | 977 |
| PPV4_F6 | AAAGCCTTTTACCGCACCCT | 3891–3910 | | 1,020 |
| PPV4_R6 | ATGTTTTCTGTGGGCCGTCA | 4891–4910 | | 1,020 |
| PPV4_F7 | CTGTGCCAGGACATCTACCG | 4527–4546 | | 1,337 |
| PPV4_R7 | ATATCATCTGCGGTGTCTGGG | 5843–5863 | | 1,337 |


https://doi.org/10.14405/kjvr.20230036

Five full genes and one VP1 gene of PPV1 from different geographical regions have been sequenced. PPV4 from different geographical regions has succeeded in sequencing 5 full genes and one ORF3 gene. The completed 12 sequences were called PPV1KUIP22-1 to 6 and PPV4KUIP22-1 to 6. All sequence information is registered in the GenBank and has received accession numbers (ON982164, OP377030-OP377035, and OP377054-OP377058).

In the case of PPV1, a premature termination codon was formed by a mutation in one of the sequences. The nucleotide at 482 of the NS2 gene of PPV1KUIP22-1 was mutated from G to A, resulting in a stop codon, and the amino acid sequence length was shortened from 162 to 161 (Fig. 1A). PPV1 shows a significant difference in pathogenicity with only one change in the amino acid [4,13]. Therefore, a premature stop codon mutation of PPV1 NS2 may show significant differences in the pathogenicity and infectivity of the virus, even though only one amino acid is shortened. In addition, a premature stop codon mutation of porcine bocavirus, which is phylogenetically close to porcine parvovirus, was observed in the previous study, and

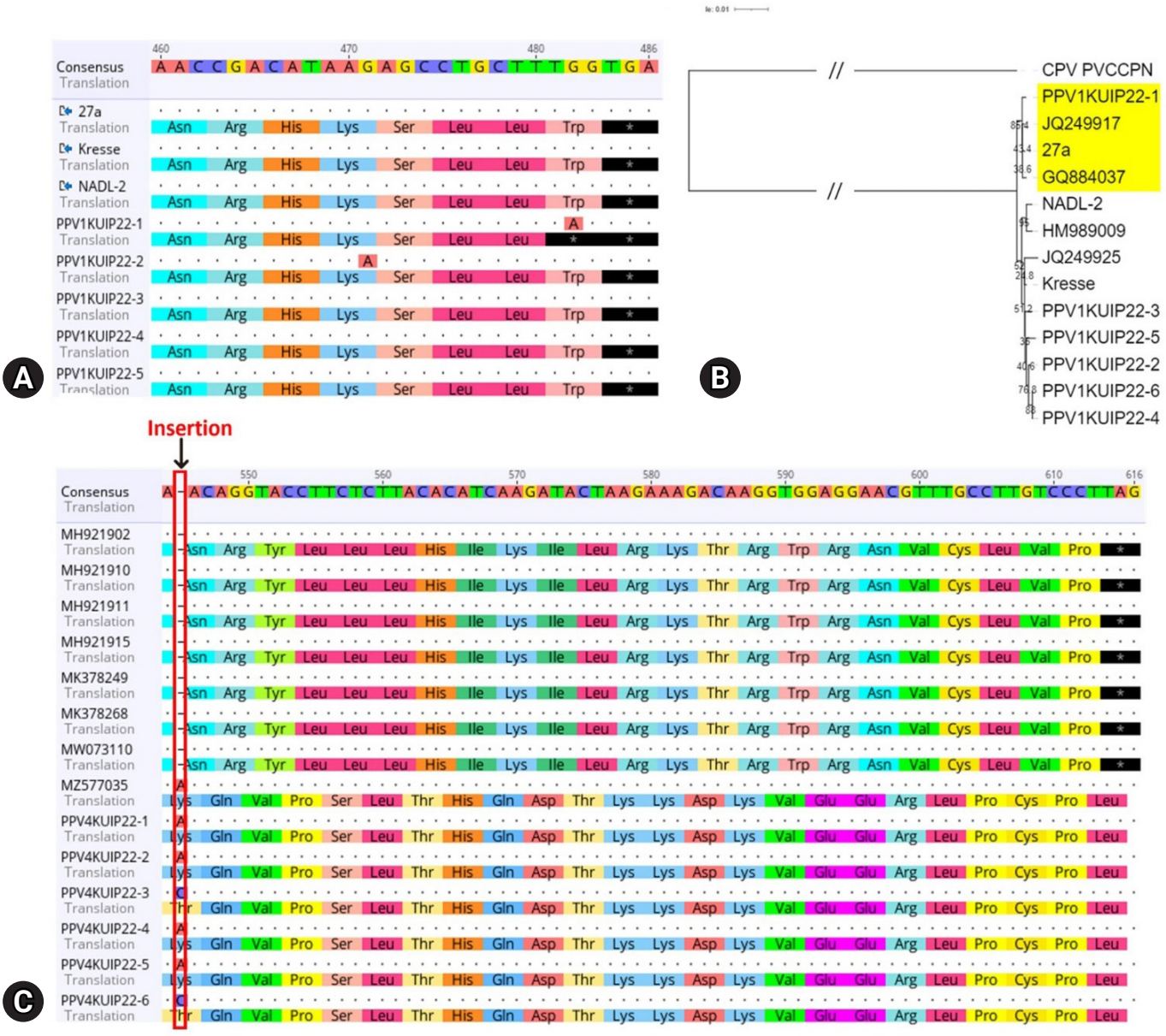


Fig. 1. Notable mutations of porcine parvovirus (PPV)1 and PPV4. Premature stop codon of NS2 (A) and phylogenetic tree based on VP1 (B) of PPV1 included. The yellow mark on the tree indicates a 27a-like strain. Canine parvovirus was used as an outgroup. NADL-2 (NC_001718), Kresse (PPU44978), and 27a (AY684871) were also used to make a tree and nodes mean bootstrap value. Frameshift mutation (C) of ORF3 of PPV4 is also indicated.

these mutations may cause a change in host specificity [14].

Phylogenetic analysis confirmed that PPV1KUIP22-1 is a 27a-like strain (Fig. 1B). The 27a strain in Germany, which has changed its neutralizing epitope because of mutation of aa 228, lacks cross-neutralization against the NADL-2 vaccine strain [4,15]. The same mutation as above was observed in PPV1KUIP22-1, and the commercial vaccine currently used in South Korea is a virus like NADL-2, such as PVK-1. No clear evidence has shown that Korean 27a-like strains are difficult to defend with existing vaccines. Nevertheless, the emergence of new PPV1 strains with different antigenicity is possible. The existence of the 27a-like PPV1 must be considered, and continuous variation monitoring through additional sampling, sequencing, antigenicity, and cross-neutralization assay with existing vaccine strain is required. Because PPV1KUIP22-1 is a 27a-like strain and has a premature stop codon at the same time, further research will be needed to determine the changes in antigenicity.

For PPV4, one nucleotide is inserted into nucleotide at 545 of ORF3 in all collected sample sequences, resulting in a frameshift mutaction (Fig. 1C). The stop codon of ORF3 disappears by this mutation, and the new stop codon is the same as that of the VP gene. This must be confirmed by virus isolation and further in vitro analysis. In most cases, frameshift mutations cause lower fitness of viruses, but some major changes have also been observed, such as host changes, immune avoidance, pathogenicity, and transmission [16]. Although the role of ORF3 in PPV4 is unknown, it may be involved in the replication of viruses [17]. The role of the protein will change significantly if a frameshift occurs and a longer protein is translated. Nevertheless, there are no successful cases of PPV4 isolation in cell culture. Therefore, further study will be needed to determine how this frameshift affects the virus after successful isolation in vitro. The possibility that ORF3 protein is a virtual protein that does not exist cannot be excluded owing to the lack of such data with the isolated virus strain.

Through the premature stop codon of PPV1 and frameshift of PPV4, an important mutation described elsewhere can be a precursor to host range change. In addition, 27a-like strains of PPV1, which show a lack of cross-neutralization against the existing vaccine strains, have been confirmed in Korea. Therefore, it is necessary to check whether the host range of PPVs and antigenicity are changed through continuous monitoring.

## Funding

This study was supported by Konkuk University's research support program for its faculty on sabbatical leave in 2022.

## Data Availability Statement

Contact the corresponding author for data availability.

## Author's Contributions

Conceptualization: Lyoo YS, Park B; Data curation: Park B, Hong J; Formal analysis: Park B; Funding acquisition: Lyoo YS; Investigation: Park B; Methodology: Lyoo YS, Park CK; Project administration: Lyoo YS; Park CK; Resources: Lyoo YS; Software: Park B, Choi AK, Jun J; Supervision: Lyoo YS; Validation: Hong J, Choi AK, Jun J; Visualization: Park B; Writing–original draft: Park B, Hong J, Jun J, Choi AK; Writing–review & editing: Lyoo YS, Park CK.

## ORCID

Beomsu Park, https://orcid.org/0009-0008-8049-3584
Jihyeon Hong, https://orcid.org/0009-0008-7217-4380
Jongsu Jun, https://orcid.org/0009-0003-3243-767X
An Kook Choi, https://orcid.org/0009-0002-5685-4905
Choi Kyu Park, https://orcid.org/0000-0002-0784-9061
Young Soo Lyoo, https://orcid.org/0000-0002-0048-1911

## References

1. Cotmore SF, Agbandje-McKenna M, Chiorini JA, Mukha DV, Pintel DJ, Qiu J, Soderlund-Venermo M, Tattersall P, Tijssen P, Gatherer D, Davison AJ. The family Parvoviridae. Arch Virol 2014;159:1239–1247.
2. Streck AF, Truyen U. Porcine parvovirus. Curr Issues Mol Biol 2020;37:33–46.
3. Mészáros I, Olasz F, Cságola A, Tijssen P, Zádori Z. Biology of porcine parvovirus (ungulate parvovirus 1). Viruses 2017;9:393.
4. Zeeuw EJL, Leinecker N, Herwig V, Selbitz HJ, Truyen U. Study of the virulence and cross-neutralization capability of recent porcine parvovirus field isolates and vaccine viruses in experimentally infected pregnant gilts. J Gen Virol 2007;88(Pt 2):420–427.
5. Cheung AK, Wu G, Wang D, Bayles DO, Lager KM, Vincent AL. Identification and molecular cloning of a novel porcine parvovirus. Arch Virol 2010;155:801–806.
6. Parrish CR, Kawaoka Y. The origins of new pandemic viruses: the acquisition of new host ranges by canine parvovi-


https://doi.org/10.14405/kjvr.20230036

rus and influenza A viruses. Annu Rev Microbiol 2005;59:553–586.
7. Lee H, Callaway HM, Cifuente JO, Bator CM, Parrish CR, Hafenstein SL. Transferrin receptor binds virus capsid with dynamic motion. Proc Natl Acad Sci U S A 2019;116:20462–20471.
8. Ma W, Kahn RE, Richt JA. The pig as a mixing vessel for influenza viruses: human and veterinary implications. J Mol Genet Med 2008;3:158–166.
9. Kim SC, Jeong CG, Nazki S, Lee SI, Baek YC, Jung YJ, Kim WI. Evaluation of a multiplex PCR method for the detection of porcine parvovirus types 1 through 7 using various field samples. PLoS One 2021;16:e0245699.
10. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 2004;32:1792–1797.
11. Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 2003;52:696–704.
12. Zimmermann P, Ritzmann M, Selbitz HJ, Heinritzi K, Truyen U. VP1 sequences of German porcine parvovirus isolates define two genetic lineages. J Gen Virol 2006;87(Pt 2):295–301.
13. Simpson AA, Hébert B, Sullivan GM, Parrish CR, Zádori Z, Tijssen P, Rossmann MG. The structure of porcine parvovirus: comparison with related viruses. J Mol Biol 2002;315:1189–1198.
14. Yoo SJ, Sunwoo SY, Ko SS, Je SH, Lee DU, Lyoo YS. A novel porcine bocavirus harbors a variant NP gene. Springerplus 2015;4:370.
15. Streck AF, Canal CW, Truyen U. Viral fitness and antigenic determinants of porcine parvovirus at the amino acid level of the capsid protein. J Virol 2022;96:e0119821.
16. Ji YF, Shao JW, Chen RX, Gong HY, Sun MH, Li GH, Chen JM. Frameshift mutations in the microevolution and macroevolution of viruses [Preprint]. Posted 2022 Jul 20. bioRxiv. https://doi.org/10.1101/2022.07.20.500745.
17. Cadar D, Cságola A, Lorincz M, Tombácz K, Kiss T, Spînu M, Tuboly T. Genetic detection and analysis of porcine bocavirus type 1 (PoBoV1) in European wild boar (Sus scrofa). Virus Genes 2011;43:376–379.