Short communication

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Running title: First identification of porcine parvovirus7 in Korea

First detection and genetic characterization of porcine parvovirus 7 from Korean domestic pig farms

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Porcine parvovirus 7 (PPV7) was first detected in Korean pig farms in 2017. The detection rate of PPV7 DNA was 24.0% (30/125) in aborted pig fetuses and 74.9% (262/350) in finishing pigs, suggesting that PPV7 is circulated in Korean domestic pig farms. Phylogenetic analysis based on VP amino acid sequences demonstrated that the nine Korean strains (PPV-KA1-3 and PPV-KF1-6) were closely related to that of previously reported US and Chinese PPV7 strains. In addition, the Korean strains have genetic diversity with insertion or deletion mutations. This study contribute to the understanding of the molecular epidemiology of PPV7 in Korea.

Keywords: porcine parvovirus 7, prevalence, domestic pigs, aborted fetus, finishing pigs

Until now, seven genotypes of porcine parvoviruses (PPV) have been identified in pig populations, which are taxonomically divided into four genera based on amino acid similarity in the NS1 protein: PPV1 in *Protoparvovirus*; PPV2 and PPV3 in *Tetraparvovirus*; PPV4, PPV5, and PPV6 in *Copiparvovirus*; and PPV7 in *Chapparvovirus* [4,5,8]. Although PPV1 is a well-known infectious agent that causes reproductive failure in swine herds worldwide, the clinical significance of other genotypes of PPV infections remains uncertain. PPV7 is the most recently identified PPV genotype and was first identified by mutagenomic sequencing from healthy adult pigs in the US in 2016 [4]. It was also detected from serum samples obtained from two Chinese pig farms with a relatively higher prevalence rate (32.8%) than that observed in the US (8.6%) [8], suggesting that PPV7 commonly circulates in pig herds in both countries. However, the clinical presentations of PPV7 infection and distribution of PPV7 in the global pig population remain to be determined. Despite growing concerns about PPV7, knowledge about the distribution of this virus in the Republic of Korea is limited. The aim of this study was to provide the first detection and genetic characterization of PPV7 from aborted pig fetuses and finishing pigs in Korean domestic pig farms.

A total of 125 aborted pig fetuses and 350 lung tissues of finishing pigs were collected from commercial pig farms located in four provinces from the Republic of Korea, which were submitted in the Viral Disease Division of the Animal and Plant Quarantine Agency for diagnosis of reproductive and respiratory disorders in 2017 (Table 1). The lung tissues of the finishing pigs and fetuses were homogenized with PBS (pH 7.2) and stored at –80ºC until use. Total DNA was extracted from the homogenized samples using the DNeasy mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. PPV7 DNA was amplified with primers targeting the *VP* gene of PPV7 (PPV7-3434-F and PPV7-3654-R) using Hotstart PCR premix (Bioneer, Daejeon, Korea) as previously described [8]. The expected 241-bp amplicons for PPV7 were confirmed. To further characterize Korean PPV7 strains, the *REP*

or *VP* genes of PPV7 were amplified using four sets of primers (PPV7-380-F and PPV7-1336-R, PPV7-1270-F and PPV7-2262-R, PPV7-2158-F and PPV7-3203-R or PPV7-3022-F and PPV7-4033-R) as previously described [8]. The amplicons were ligated into the pDrive vector (Qiagen, Hilden, Germany) and sent to a commercial sequencing company (Macrogen, Seoul, Korea) for sequencing of the *REP* or *VP* gene. The sequences were assembled using the SeqMan program in Lasergene 12.0 software (DNASTAR, Inc., Madison, Wisconsin, US) and aligned with other PPV sequences downloaded from GenBank by Clustal Omega (http://www.ebi.ac.uk/). Phylogenetic trees were inferred from amino acid sequences of the VP protein by the maximum-likelihood method using the Le and Gascuel with gamma distributed rate variation and frequency of each amini acid (LG+F+I) model implemented in MEGA v6.06. Support for individual nodes was determined by 1,000 bootstrap replicates [2,6].

PPV7 DNA was detected in 30 of the 125 tested fetal samples and 262 of the 350 tested finishing pig samples (Table 1). The prevalence of PPV7 in aborted pig fetal samples (24.0%, 30/125) was higher than the prevalence observed in the US (8.6%) but lower than that detected in China (32.8%). In addition, the prevalence of PPV7 in finishing pig lung tissue samples (74.9%, 262/350) was higher than the prevalence observed in the US (8.6%) and China (32.8%) [4,8]. These results suggest that PPV7 commonly circulates in Korean pig farms and that PPV7 infection may be associated with reproductive failure in breeding pigs. In this regard, additional studies, such as virus isolation and artificial infection with virus isolates are needed to elucidate the pathogenesis and clinical presentation of PPV7 in pigs. To further characterize the Korean PPV7, *VP* genes of three aborted pig fetus strains (designated as PPV7-KA1-3) and *REP* and *VP* genes of six finishing pig strains (designated as PPV7-KF1–6) were analyzed and deposited in GenBank under the accession numbers MH293507 (PPV7-KA1), MH293508 (PPV7-KA2), MH293509 (PPV7-KA3), MH422962 (PPV7-KF1),

MH422963 (PPV7-KF2), MH422964 (PPV7-KF3), MH422965 (PPV7-KF4), MH422966 (PPV7-KF5) and MH422967 (PPV7-KF6), respectively. The *VP* genes of PPV7-KA3, -KF2, -KF6 were 1410-bp in nucleotide length, which was consistent with a previous report of the US PPV7 42 strain (GenBank No. KU563733) and Chinese GD 2014-1 (GenBank No. KY996756); it was 9-bp longer than Chinese GD 2014-2 (GenBank No. KY996757) and GD 2014-3 (GenBank No. KY996758). In contrast, the nucleotide length of the other four isolates (PPV7-KA1, 2 and PPV7-KF1, 4) were 1425-bp, which is longer than that observed in US and Chinese strains owing to the successive insertion of five amino acids (181–185 amino acids). In addition, the nucleotide length of the remaining two isolates (PPV7-KF3 and PPV7-KF5) were 1404-bp and 1392-bp, respectively, which are shorter than that observed in US strain due to the deletion of two amino acids (140-141 amino acids) or two and four amino acids deletions (140-141 and 147-150 amino acids) [4,8]. Based on the sequence analysis of the *VP* genes, nine Korean PPV7 strains shared the following nucleotide and amino acid level identities with each other: 88.1%–97.9% and 88.2%–99.5% with each other, 89.8%–98.2% and 90.5%–99.3% with the US PPV7 42 strain, and 87.5%–97.8% and 85.2%–98.2% with the Chinese PPV7 strains, respectively [4,8]. The conserved calcium binding loop (YXGXG) motif was identified in the VP protein of Korean PPV7 isolates. However, the catalytic residues (HDXXY) of the putative secretory phospholipase A2 (PLA2) were missing due to a single amino acid mutation at amino acid (aa) 304 (Y to N) in the VP protein of PPV7 strains such as the US and Chinese strains [7,8]. Phylogenetic analysis based on VP amino acid sequences demonstrated that the nine Korean strains were closely related to previously reported US and Chinese PPV7 strains, and they clustered in the *Chapparvovirus* group together with turkey parvovirus (GenBank No. KR925531) (Fig. 1), which is consistent with a previously performed phylogenetic analysis based on the NS protein [4,8]. In addition, Korean PPV7 strains have various mutations comparing US and Chinese strains (Fig. 1).

These results indicate that Korean PPV7 has more genetic diversity than US and Chinese PPV7. PPV1 was prevalent in Korean pig farms and is continuously surveyed for owing to significant economic costs it has had for the swine industry [3]. Additionally, PPV2 was also recently isolated from pigs in 2016 [1]. However, PPV7 infection has not yet been reported in the Republic of Korea. To the best our knowledge, this is the first report for PPV7 detection from aborted pig fetuses and finishing pigs in the Republic of Korea. The results of this study contribute to the understanding of the molecular epidemiology of PPV7, and further studies using PPV7 isolates will be needed to elucidate the pathogenesis of this virus in pigs.

Acknowledgments

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Conflict of Interest

The authors declare no conflicts of interest.

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Table 1. PPV7 DNA detection rates in aborted pig fetuses and finishing pigs

Province/area	Aborted pig fetuses	Finishing pigs
Positive	30	262
Negative	95	88
Total	125	350
Positive rate	24.0%	74.9%

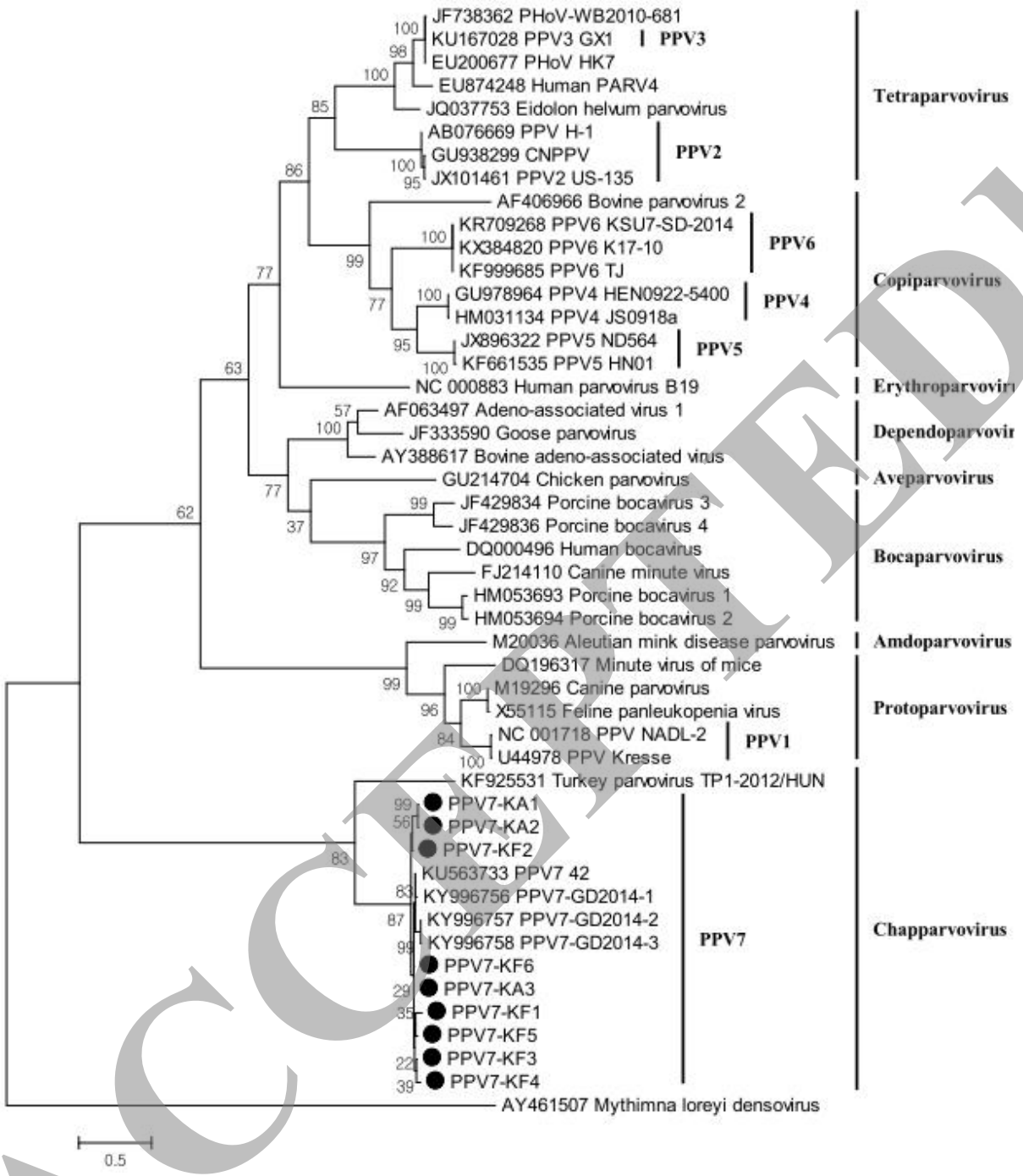

Fig. 1. Phylogenetic tree constructed based on VP protein sequences. Phylogenetic tree of VP protein sequences derived from 39 *Parvovirinae* genomes. The tree was inferred from amino acid sequences of the VP protein by the maximum-likelihood method using the LG+F+I model under 1,000 bootstrap resampling iterations. The Korean PPV7 strains identified in this study are represented by black circles.