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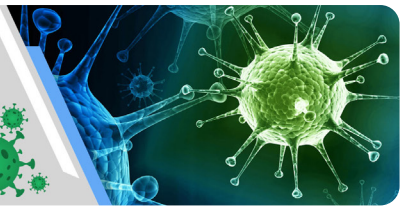

Prevalence of Porcine Parvovirus 1–6 Detected in South Korean Domestic Pigs

In-Ohk Ouh[1,2], Seo Young Moon[1], Ju-Yeon Lee[1], Seong-In Lim[1], Yeun-Kyung Shin[1], Dongmi Kwak[3], Yoon-Hee Lee[*,1], Choi-Kyu Park[*,3]

Abstract

Porcine parvovirus (PPV) 1 is a major causative pathogen of reproductive failure and PPV2–6 were recently identified as newly emerging viruses with unknown pathogeneses. In this study, we examined 926 samples from domestic pig farms for the presence of PPV1–6 co-infection. The prevalence of PPV1–6 was significantly greater in the lung tissue samples than in the aborted pig fetus samples. PPV3–6 were detected for the first time in Korean domestic pigs, and concurrent infections were more common than single infections in the pig population with numerous infectious pathogens such as PPV. Furthermore, the Korean PPV1 strain (PPV1-82) was identical to the virulent PPV-27a strain in this study. These results describe, for the first time, the prevalence of PPV1–6 in South Korean domestic pigs.

Keywords: Pig, Porcine parvovirus 1–6, Prevalence, Genetic diversity

Introduction

Porcine parvoviruses (PPVs) are small, non-enveloped, single-stranded linear genome DNA viruses of 4–6.3 kb [1]. PPVs are influential pathogens that can induce reproductive failure in pigs, which results in massive economic losses in the pig industry worldwide [2]. During the last two decades, several novel PPVs have been reported [2–9]. According to the International Committee on the Taxonomy of Viruses classification, to date, seven species of PPV have been discovered in pigs, belonging to four genera based on the similarity of the non-structural protein 1 (NS1): protoparvovirus (PPV1), tetraparvovirus (PPV2–3), copiparvovirus (PPV4–6), and chap parvovirus (PPV7) [9,10]. PPV2 shares a high genetic similarity with parvoviruses detected in Chinese pigs with clinical symptoms that include post-weaning multisystemic wasting syndrome (PMWS) and high fever [5,7] and with parvoviruses that were first found in swine sera in Myanmar [4]. PPV3 was identified in Hong Kong in 2008 and was initially called porcine norovirus (PHoV). However, phylogenetic analyses and comparative sequencing indicated that PHoV was more similar to the newly described bovine norovirus and human parvoviruses 4 and 5, which form a distinct cluster within parvoviruses [11]. Phylogenetic analysis revealed that PPV4–6 were closely related and formed a distinct branch [8,12]. The prevalence of PPV2–6 has not been determined in Korea. Therefore, in this study, we investigated the prevalence of PPV1–6. Furthermore, we characterized the genomes of PPV1–6 and analyzed their phylogenetic trees, comparing the results of these newly detected viruses with those of other reported strains in Korea and elsewhere.

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Materials and Methods

Sample preparation

The prevalence of PPV1–6 was assessed from 926 samples that were obtained from 215 farms, which included 272 aborted pig fetus-mixed tissue samples from 136 farms located in six different provinces and 654 lung tissue samples from 79 domestic pig farms that were obtained from abattoirs in five different provinces over 2.5 years from 2017 to 2019 (Supplementary Table S1). Among these, aborted pig fetuses were sought for pathogen differential diagnosis, and domestic pigs aged five to six months were randomly selected from abattoirs. These samples were submitted to the Viral Disease Division of the Animal and Plant Quarantine Agency for the diagnosis of porcine reproductive failure diseases, post-weaning multisystemic wasting syndrome (PMWS), porcine circovirus associated disease (PCVAD), and porcine respiratory disease complex (PRDC) (OPTI-IAC-2001).

DNA extraction

Samples were homogenized using a Precellys® CK28-R Lysing kit with a bead tube for hard tissue homogenization (Bertin Technologies, Bretonneus, France) and a Precellys® evolution homogenizer (Bertin Technologies). Total DNA was extracted using a commercial DNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

Molecular detection of viral nucleic acids

Individual PCR amplification for each pathogen was performed using an Accu-Power HotStart PCR Premix Kit (Bioneer, Daejeon, Korea) with specific primers for the detection of PPV1–6 and PCV2 and 3 [3,13,32–37]. Positive samples were PCR amplified for near-complete major capsid gene sequencing of PPV1 and near-complete sequencing of PPV2–6. When no signal was observed after 30 cycles of amplification, the sample was considered negative. All primers and amplification conditions used in this study are described in Supplementary Table S2.

DNA sequencing and phylogenetic analysis

Further sequencing of the near-full-length major capsid genes was conducted to evaluate genetic differences between the strains. Target genes were amplified using the amplification sequencing primers listed in Supplementary Table S2. Positive amplicons were purified using an agarose gel extraction kit (Qiagen) and ligated into the pDrive vector (Qiagen) according to the manufacturer's instructions. The ligation products were transformed into Escherichia coli DH5 α-competent cells and incubated overnight at 37°C. The ligated vectors were extracted using a plasmid minipress kit (Inclone, Daejeon, Korea) and sequenced using Macrogen vector-sequencing primers (Macrogen Inc., Seoul, Korea). The sequences obtained were identified using the National Center for Biotechnology Information's Basic Local Alignment Search Tool (BLAST) [38]. Multiple sequence alignments and homologies of the nucleotide sequences of the PPV1–6, PCV2, and PCV3 isolates were performed using the CLC Main Workbench software (v. 7.0.3; CLC Bio, Qiagen). The sequence alignment results were modified using BioEdit v. 7.2.5 (https://bioedit.software.informer.com/7.2/, accessed on March 1, 2021) and analyzed using a similarity matrix. Phylogenetic analysis was performed using MEGA v. 6.0 (https://megasoftware.net/, accessed on March 1, 2021), and the reference sequences of the major capsid genes [39] were obtained from the GenBank database (Supplementary Table S3), and the maximum-likelihood approach with 1,000 bootstrap replicate values was applied.

Statistical analysis

Pearson's chi-square test was used to determine the significance of intergroup differences. The analytical software package GraphPad Prism (v. 5.04; GraphPad Software Inc., La Jolla, CA, USA) was used for all statistical calculations. Statistical significance was set at a p-value < 0.05, and 95% confidence intervals (CI) were calculated.

The nucleotide sequences of PPV1–6 obtained in this study were deposited into the GenBank database with the following accession numbers: PPV1 (MH447542–MH447550 and MZ856459), PPV2 (MH921914), PPV3 (MZ856460), PPV4 (MH921902, MH921910, MH921911, MH921915), PPV5 (MH21904, MH921905, MH921908, MH921912, MH921913), and PPV6 (MH447535–MH447541) (Supplementary Table S4).

Results

Prevalence of the PPV and PCV viruses

Of the 926 samples tested, PPV4 was the least prevalent of the PPV and PCV viruses, whereas PPV2 was the most prevalent. The prevalence of viruses in the aborted fetuses was consistently lower than that in the lung tissue samples (Table 1). The individual prevalence of these viruses was low, ranging from 0.7% for PPV4 (6/926, 95% CI: 0.1–1.2) to 4.21% for PPV1 (39/926, 95% CI: 2.9–5.5) (Supplementary Table S5).

Prevalence of PPV1 co-infection with PPV2–6

PPV1 co-infection with PPV2–6 was present to a similar degree with all viruses, ranging from 22.9% with PPV4 (212/926, 95% CI: 20.2–25.6) to 34.8% with PPV2 (322/926, 95% CI: 31.7–37.8) (Table 2). Co-infection with PPV1 in the aborted pig fetuses was low and was present in 1.1–5.9% of infections, whereas this was greater in the lung samples, ranging from 32.0–47.2%.

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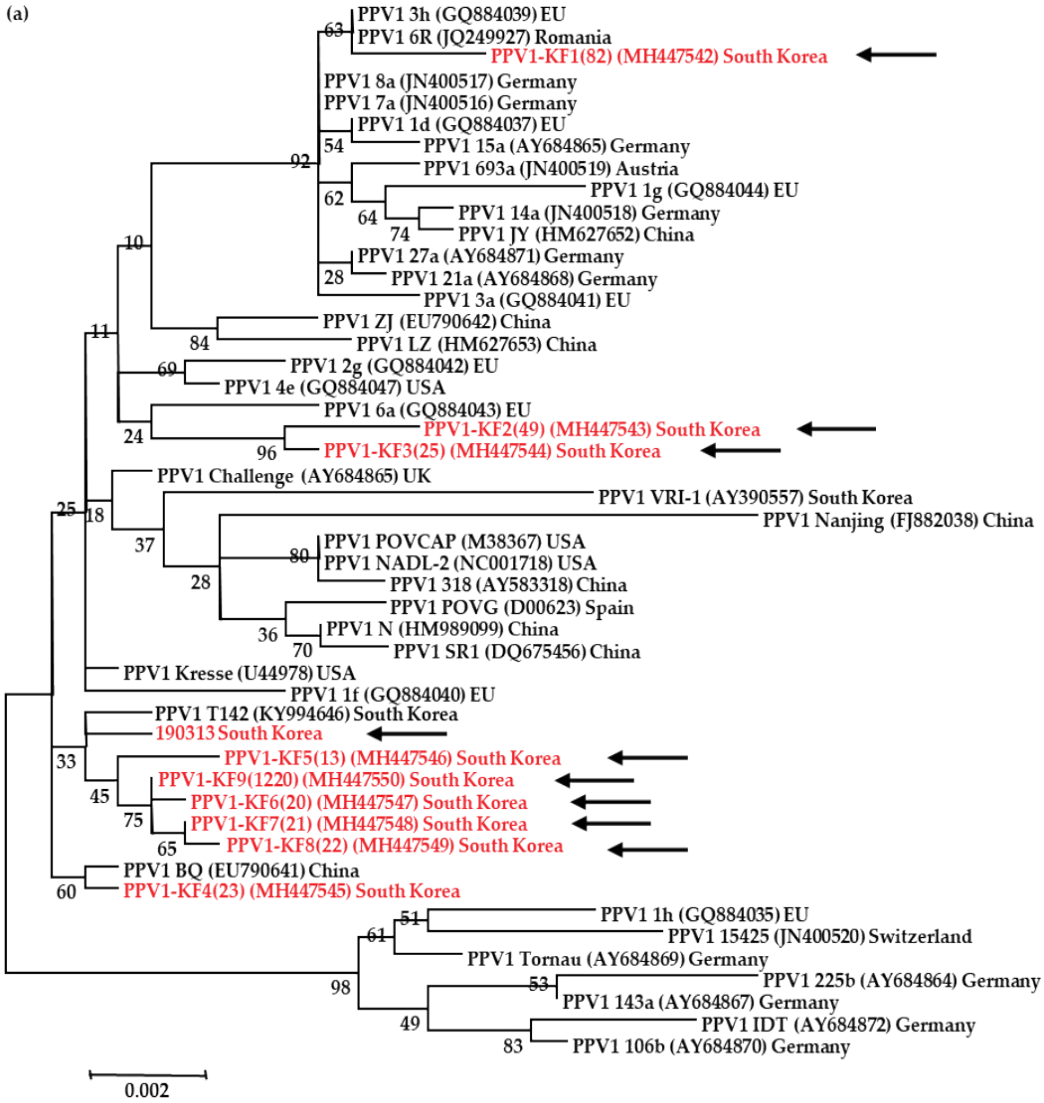
Genetic analyses of the sequenced genomes

Sequencing of the near full-length major capsid genes (PPV1, 1740–1749 bp) and near full-length genomes (PPV2–6, 5350–5979 bp) was performed to evaluate the possibility of genetic variations between the strains. Among the positive samples, representative samples were selected for phylogenetic analysis. Therefore, ten PPV1, one PPV2, one PPV3, four PPV4, four PPV5, and seven PPV6 positive samples were selected. These strains were classified as either VP2 (PPV1) or VP1 (PPV2 to 6) through amino acid-based phylogenetic tree analysis (Figure 1). We detected ten PPV1 strains and sequenced each VP2 gene (designated as 13, 20, 21, 22, 23, 25, 49, 82, 1220, and 190313). Nine sequences of the VP2 genes of PPV1 were detected in the abattoir samples (13, 20, 21, 22, 23, 25, 49, 82, and 1220) and one in the aborted pig fetuses (190313). Amino acid similarities between the sequenced VP2 genes and the NADL-2 strain (GenBank acc. no. NC001718) ranged from 98.1–100%. Phylogenetic analysis revealed that the Korean PPV1-82 strain in the abattoir samples clustered with the highly virulent PPV-27a strain (GenBank acc. no. AY684871) (Figure 1a). Next, we detected and sequenced one PPV2 strain from the near full-length genome of PPV2 in the abattoir samples. The Korean PPV2 sequence exhibited high amino acid similarity (98.5% in NS1 and 96.2% in VP1) with a Chinese strain (GenBank acc. no. KU745627). Parvoviruses genetically similar to the Korean PPV2 strain have been detected in China (Figure 1b). One sequence of the near full-length genome of PPV3 was detected in the abattoir samples. Regarding the PPV3 phylogenetic tree, the complete major capsid genes were compared with those of the initial isolates from Hong Kong, and the results revealed that the Korean PPV3 capsid protein was highly conserved (Figure 1c). Korean PPV3 sequences showed a high amino acid similarity (98.1% in NS1 and 98.8% in VP1) with the Hong Kong strain (GenBank acc. no. EU200673).



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(b)

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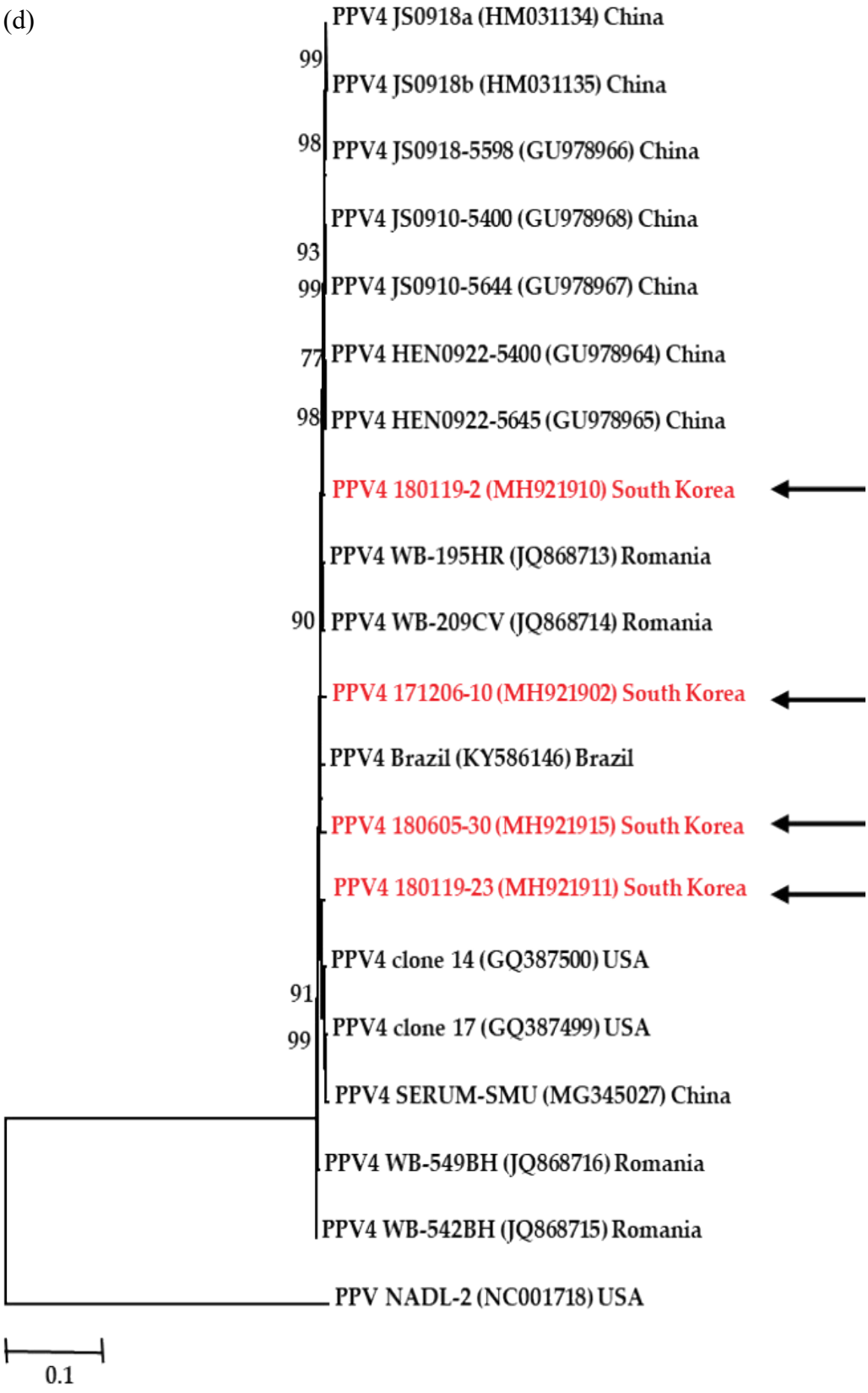

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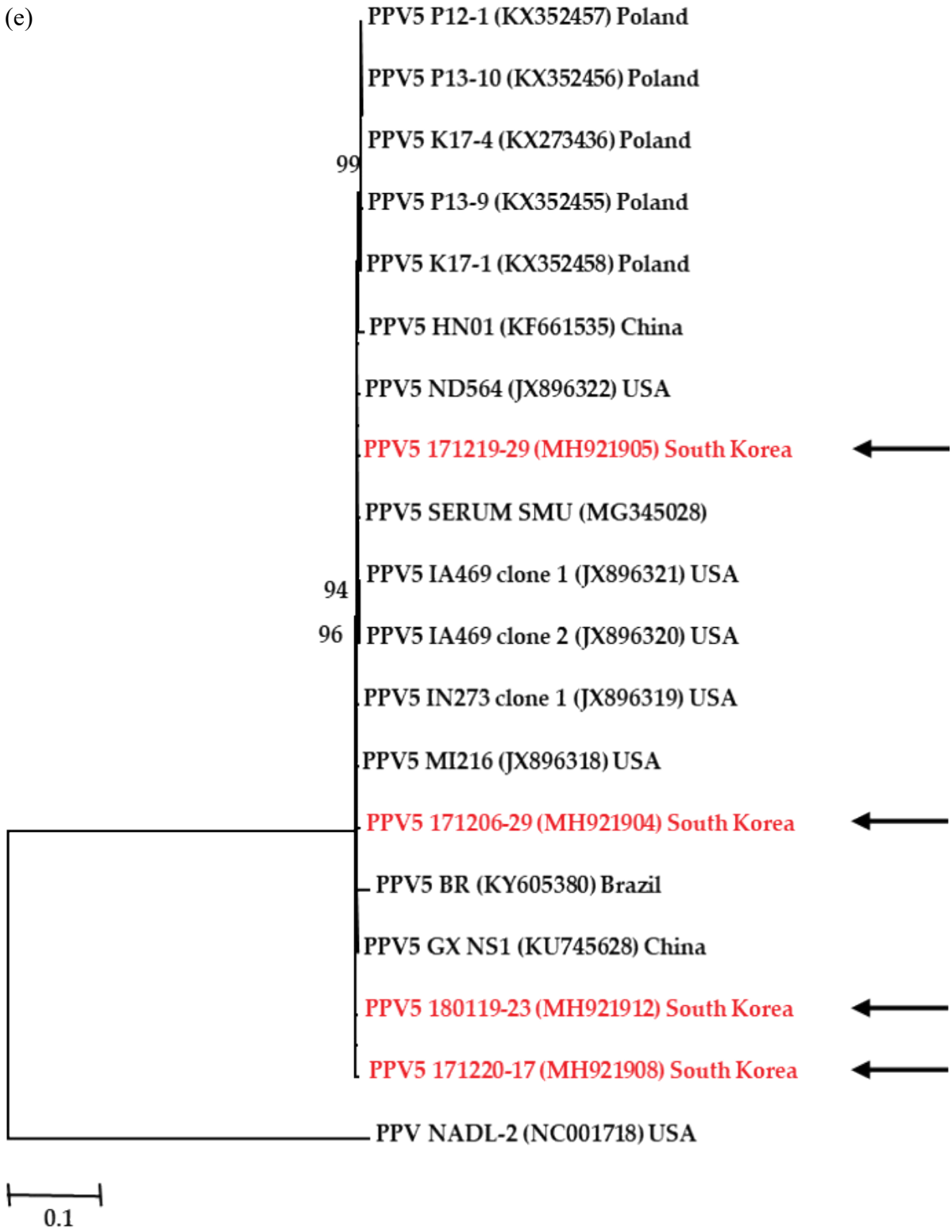

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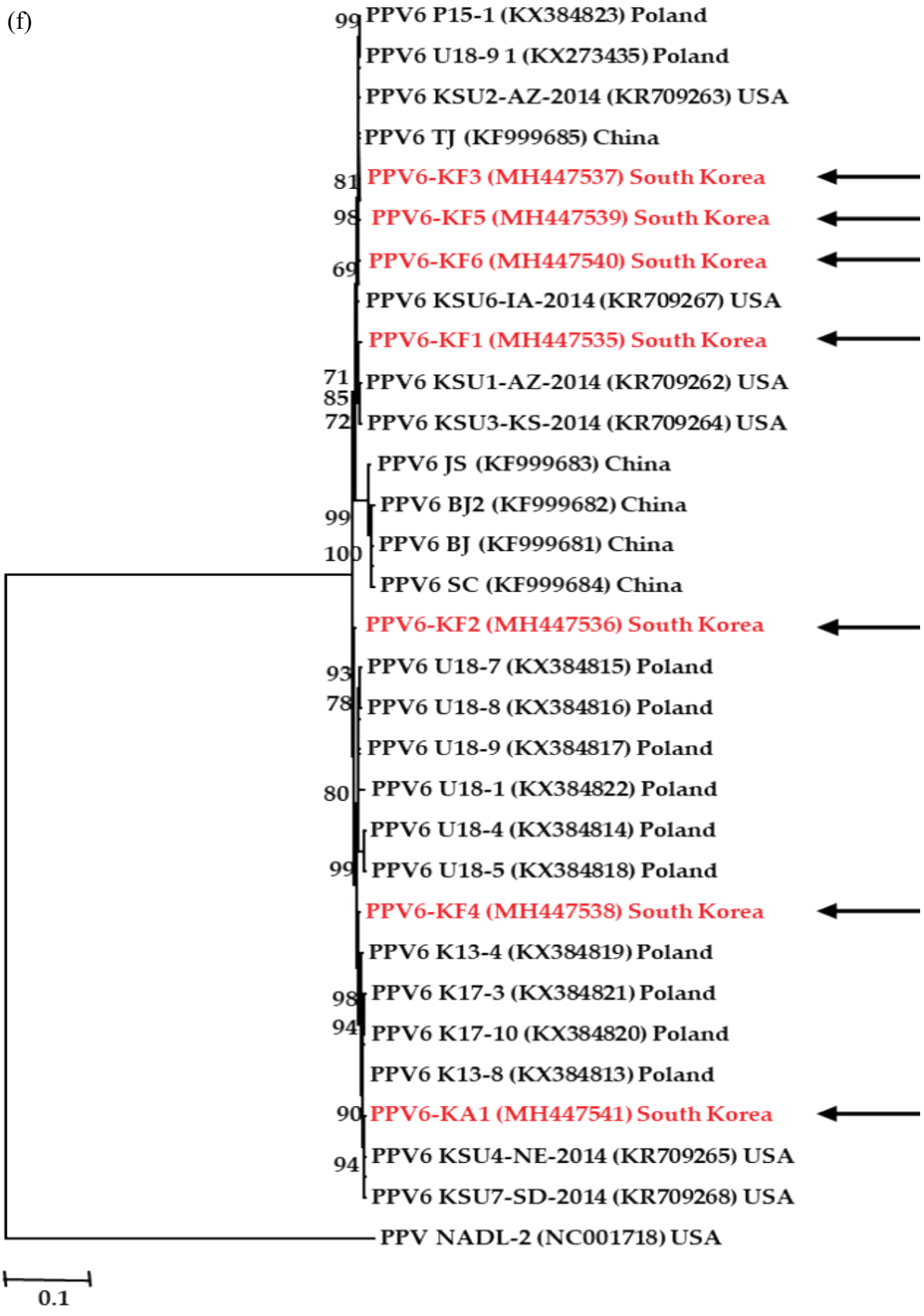

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Four sequences of near full-length PPV4 genomes were detected in two abattoir samples and two aborted pig fetuses (Figure 1d). Two PPV4 sequences (S180119-2 and S180119-23) in aborted pig fetuses exhibited 98.3% and 96.8% amino acid identity with a Chinese strain (GenBank acc. no. GU978965) and a US strain (GenBank acc. no. GQ387500), respectively. In addition, two PPV4 sequences (S171206 and S180605) in the abattoir samples exhibited high nucleotide identity (96.5% and 97.6%) with a Chinese strain (GenBank acc. no. GU978965) and a Brazilian strain (GenBank acc. no. KY586146), respectively. Near full-length genomes of four novel Korean PPV5 strains were detected in three abattoir samples and one aborted pig fetus. Phylogenetic analysis based on VP1 amino acid sequences demonstrated that the four Korean PPV5 strains were closely related to a previously reported US strain (GenBank acc. no. JX896319). In addition, these four Korean PPV5 strains were closely related to each other and the US strain at the nucleotide level (99.2%–99.4%) (Figure 1e). In PPV6, the near full-length genomes of seven novel strains were detected in six abattoir samples and one aborted pig fetus sample. The near full-length genomes of four strains from the abattoir lung tissue samples shared 98.5%–99.5% amino acid sequence identity with a Chinese PPV6 strain (GenBank acc. no. KF999685). Near full-length genomes of one PPV6 strain that was discovered from the lung tissue and one PPV6 strain that was discovered in the aborted pig fetuses shared 96.0% and 99.3% amino acid sequence identity with a US PPV6 strain (KR709266), respectively. The other PPV6 strains identified from the abattoirs shared 98.2% amino acid sequence identity with a Brazilian PPV6 strain (GenBank acc. no. KY094494). Phylogenetic analysis of the VP1 genes indicated that the PPV6 strains clustered into three distinct groups (Figure 1f).

Discussion

The prevalence of PPV1–7 in Europe varies widely [32,40-44]. Here, we examined the molecular characteristics of the PPV1–6 strains circulating in domestic pigs in Korea and reported their detection rates and genetic characteristics. Notably, PPV3–6 were detected for the first time in Korean domestic pigs. We found that the prevalence of PPV in lung tissue samples sourced from abattoirs was significantly greater than that found in the aborted pig fetus samples. PPV1 has previously been detected in Korea, but only in 1 of the 701 samples collected from 2013 to 2016 [45]. PPV2 has been detected in many other countries, including Hungary, the USA, Japan, Germany, and Thailand at rates of 6%, 21%, 58%, 78%, and 83%, respectively [46–50], and in 2016, PPV2 was detected in two lung tissue samples in Korea [51]. However, PPV3–6 have not been previously detected in Korean domestic pigs. In this study, the prevalence of PPV6 among the aborted pig fetal samples (18.0%) was lower than that observed in China (50.0%), and the prevalence of PPV6 in the abattoir samples (60.9%) was higher than that reported in China (15.6%) [8]. In 2017, we detected the prevalence of PPV7 in aborted pig fetuses (24%) and domestic pigs (74.9%) in Korea [52]. In this study, we only detected unique PPV1–6 infections in a minority of aborted pig fetus samples. The genome sequences and phylogeny analyses indicated that the Korean strains were closely related to strains circulating in the US, Brazil, and Hong Kong, China. Novel PPVs, such as PPV4, PPV6, and PPV7, are suspected to cause reproductive failure because they have been detected in aborted pig fetus samples [8,46,52]. Furthermore, PPV4 and PPV6 have been identified in adult female pigs with reproductive disorders [8,53]. In this study, these pathogens (PPV1–6) were all uniquely detected in the aborted pig fetuses. The role of these viruses in reproductive failure disease pathogenesis and epidemiology needs to be studied in detail because of their current prevalence in major swine populations worldwide. Previous studies have detected PPV4/PPV5 co-infection in 15.6% of lung samples from infected pigs [3].

In this study, co-infection of PPV1 with PPV2, 3, 4, 5, or 6 was detected in Korean domestic pigs for the first time, and the abattoir samples had considerably higher co-infection rates than the aborted pig fetus samples. Co-infections are more common than single infections in swine populations, and several infectious pathogens, such as PPV, can influence respiratory diseases [54]. Furthermore, co-infection with PPV1 and PCV2 enhances PCVAD severity and pathological lesions in the lymphoid tissues [55]. Interestingly, PPV2 viremia was detected 2–3 weeks before the presence of respiratory signs and the development of clinical PCVAD symptoms [5]. Because lung samples were randomly collected from the abattoirs, no additional information was available regarding the disease status of the animals from which the studied lungs originated. Therefore, the results show the prevalence of the studied viruses in the population but do not indicate the role of any of these viruses in the development of respiratory disease. A previous study proposed that PPV1 and PPV7 may increase the severity of PCV2 subclinical infections in fatteners through the excitation of PCV2 replication, which may lead to PCVAD in individuals. The mechanism underlying the influence of PPV1 on PCV2 infection is well documented, whereas the pathogenesis of PPV7 remains to be elucidated [40]. To address this, further studies are required under controlled conditions to determine the pathogenic co-infection rates of PPV1 with PPV2–6 and PCV3. Genetically, the PPV1-82 strain from domestic pigs clustered with the virulent PPV-27a strain. Cross-neutralization studies conducted against the vaccine strains IDT and NADL-2, which exhibit low neutralization activity against the PPV-27a strain, indicated that the existing PPV1 vaccine was ineffective in preventing the spread of PPV1 [67,68]. Currently, inactivated vaccines

are based on NADL-2 and related strains, which were isolated approximately 30 years ago. These vaccines are effective against homologous infections, although they do not prevent viral shedding or infection after being challenged with the antigenically heterologous PPV-27a strain [68]. Infection of pigs with PPV-27a or injection of the virus into rabbits resulted in homologous neutralizing antibody titers that were 100- to 1,000-fold lower than heterologous titers against the NADL-2, 143a, or MSV strains [67]. The presently available vaccines appear to be suitable for the protection of individual pigs against PPV disease. In this study, except for the PPV1-82 strain, the detected PPV1 strains did not cluster with PPV1-27a, thus indicating that many strains are distributed within the Korean pig population. Therefore, further research should be conducted to develop effective PPV1 vaccine candidate strains. Although the significance of amino acid substitutions has not been determined, amino acid changes in PPV1 VP1 have been reported to be responsible for the pathogenicity of the PPV1 Kresse strain [69]. It is unknown whether the amino acid variability in PPV6 VP1 affects the pathogenicity or tissue tropism of the virus [12]. Nevertheless, this is the first study to determine the molecular characteristics of the PPV3–6 strains circulating in the tissue samples from abattoirs or aborted pig fetuses.

Conclusion

In conclusion, we report here the prevalence of PPV1–6 co-infection in lung tissue samples from abattoirs and aborted pig fetuses. This study improves our understanding of these viral infections in domestic Korean pigs. The results of this study are important for the investigation of the prevalence of diseases in pork production and the improvement of biosecurity levels at the farm level in Korea. Multiple co-infections with these viruses were commonly detected, and the near full-length sequences of the major capsid genes enabled us to compare the amino acid sequences between strains and perform comprehensive phylogenetic analysis.

Data availability statement

Data supporting the conclusions of this article are included within the article. The newly generated sequences were submitted to the GenBank database under the accession numbers MH447535-MH447550, MZ856459-MZ856460, MH921902, MH21904-MH921905, MH921908, MH921910-MH921915, and MK006032-MK006039. The datasets used and/or analyzed during this study are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare no conflict of interest.

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