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Genomic and antigenic characterization of porcine epidemic diarrhoea virus strains isolated from South Korea, 2017

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Summary

Porcine epidemic diarrhoea virus (PEDV) is a globally emerging and re-emerging enteric coronavirus in pigs causing serious economic threats to the world swine industry. Since the re-emergence of massive PEDV outbreaks in South Korea in 2013–2014, domestic pig farms have continued to experience PED epidemics or endemics. This study represents the molecular characterization of PEDV isolates identified in diarrhoeic animals collected across the country in 2017. Initial sequencing analysis of the full-length S genes revealed that 70% of the 2017 isolates (7/10) belong to the G2b subgroup, while the remaining isolates were classified as G1b. The data indicated that both variant G1b and global epidemic G2b strains were responsible for current PED outbreaks in South Korea. The 2017 G1b and G2b isolates shared 98.7%–99.4% and 98.1%–99.2% amino acid sequence identity at the S gene level and 99.3% and 99.0%–99.6% nucleotide sequence homology at the genome level compared to the corresponding Korean prototype G1b and G2b strains, respectively. In an interesting manner, one G2b-like KNU-1705 strain was found to possess a large 39-nucleotide deletion in the ORF1a region theoretically encoding nonstructural protein 3. Phylogenetic analysis based on the entire genome and spike protein sequences indicated that the 2017 isolates were most closely related to other global G1b or G2b strains but formed different branches within the same genogroup. These results indicate that PEDVs undergo continuous evolution in the field. In addition, one 2017 PEDV strain, KOR/KNU-1705/2017, was successfully isolated and propagated in Vero cells. The antisera raised against the Korean prototype 2014 G2b strain efficiently neutralized KNU-1705 virus infection, suggesting antigenic homology between the 2014 and 2017 PEDV strains. Our data advance the understanding of the molecular epidemiology and antigenicity of PEDV circulating in South Korea.

KEYWORDS
antigenicity, full-length genome, phylogenetic analysis, porcine epidemic diarrhoea virus, prevalence

1 | INTRODUCTION

Porcine epidemic diarrhoea (PED) is a highly contagious, deadly enteric viral disease in pigs with economically effects on pig production. The disease causes severe watery diarrhoea, followed by fatal dehydration, and a high mortality rate in neonatal piglets (Lee, Kim, & Lee, 2015). The causative agent, PED virus (PEDV), is a large, enveloped, single-stranded, positive-sense RNA virus belonging to the genus *Alphacoronavirus* within the family *Coronaviridae* of the order *Nidovirales* (Cavanagh, 1997; Gorbalenya, Enjuanes, Ziebuhr, & Snijder, 2006; Pensaert & Debouck, 1978; Saif, Pensaert, Sestack, Yeo, & Jung, 2012). The PEDV genome is approximately 28 kb in length with a 5′ cap and 3′

polyadenylated tail and consists of seven canonical coronaviral genes, including open reading frame (ORF) 3, in the following conserved order: 5′ untranslated region (UTR)-ORF1a-ORF1b-S-ORF3-E-M-N-3′ UTR (Kocherhans, Bridgen, Ackermann, & Tobler, 2001). The first two large ORFs, ORF1a and 1b, encompass the 5′-proximal two-thirds of the genome and code for nonstructural proteins (nsps). ORF1a translation yields a replicase polyprotein (pp) 1a, whereas ORF1b is expressed by a −1 ribosomal frame shift that C-terminally extends pp1a into pp1ab. These pp1a and pp1ab are proteolytically matured by internal viral proteases to generate 16 processing end-products, named as nsp1–16. The remaining ORFs in the 3′-proximal region of the genome encode four canonical structural proteins, spike (S), envelope (E), membrane (M) and nucleocapsid (N), as well as one accessory gene, ORF3 (Duarte et al., 1994; Kocherhans et al., 2001; Lai, Perlman, & Anderson, 2007; Lee, 2015; Saif et al., 2012).

Porcine epidemic diarrhoea was first observed in feeder and fattening pigs in England in 1971 (Oldham, 1972) and caused widespread epidemics in multiple swine-producing countries in Europe during the 1970s (Opriessnig, 2016). A marked decrease in acute PED epizootics occurred in Europe in the 1980s and 1990s, and only sporadic outbreaks have occurred in recent years (Opriessnig, 2016). In Asia, PED was first reported in 1982, and unlike in Europe, it has since posed a huge economic threat to the Asian pork industry (Chen et al., 2008; Kweon et al., 1993; Li et al., 2012; Puranaveja et al., 2009; Takahashi, Okada, & Ohshima, 1983). In May 2013, PED outbreaks suddenly appeared in the United States and swiftly spread across the nation, as well as to adjacent countries. This outbreak caused the death of more than 8 million newborn piglets in the United States alone during a 1-year-epidemic period, leading to annual losses in the range of $900 million to $1.8 billion (Langel, Paim, Lager, Vlasova, & Saif, 2016; Mole, 2013; Ojkic et al., 2015; Stevenson et al., 2013; Vlasova et al., 2014). The US emergent strain-like viruses further reached East Asian countries, resulting in nationwide PED disasters (Lee, 2015; Lee & Lee, 2014; Lin et al., 2014; MAFF, 2018). During the 2013–2014 pandemics, PED rapidly swept across mainland South Korea and Jeju Island, killing hundreds of thousands of piglets in domestic herds (Lee & Lee, 2014, 2017; Lee & Lee, 2014). Since then, PED epizootics or enzootics have regionally occurred through provinces in South Korea with intensive swine industries. To investigate the diversity of PEDVs responsible for the ongoing outbreaks in South Korea, in this study, we determined the full-length sequences of the S proteins of field isolates and complete genome sequences of representative strains identified throughout 2017. In addition, we isolated and serially propagated a KOR/KNU-1705/2017 strain and assessed the antigenic cross-reactivity between 2014 and 2017 PEDV field isolates.

2 | MATERIALS AND METHODS

2.1 | Clinical sample collection

The small intestine or stool specimens were collected from piglets showing acute watery diarrhoea at various swine farms located in eight different provinces from March through December 2017. Intestinal homogenates were prepared as 10% (wt/vol) suspensions in phosphate-buffered saline (PBS) using a MagNA Lyser (Roche Diagnostics, Mannheim, Germany) by three repetitions of 15 s at a speed of 8,000 *g*. Faecal samples were also diluted with PBS to 10% (wt/vol) suspensions. The suspensions were then vortexed and centrifuged for 10 min at 4,500× *g* (Hanil Centrifuge FLETA5, Incheon, South Korea). The clarified supernatants were initially subjected to RT-PCR using a TGE/PED Detection Kit (iNtRON Biotechnology, Seongnam, South Korea) according to the manufacturer's instructions. PEDV-positive samples were filtered through a 0.22-μm-pore-size syringe filter (Millipore, Billerica, MA) and stored at −80°C until subsequent sequencing analysis and virus isolation.

2.2 | Nucleotide sequence analysis

The S glycoprotein gene sequences of the virus isolates were determined by the traditional Sanger method. Two overlapping cDNA fragments spanning the entire S gene of each isolate were amplified by RT-PCR as described previously (Lee, Park, Kim, & Lee, 2010). The individual cDNA amplicons were gel-purified, cloned into a pGEM-T Easy Vector System (Promega, Madison, WI) and sequenced in both directions using two commercial vector-specific T7 and SP6 primers and gene-specific primers. The full-length S sequences of 10 PEDV, designated KNU-1701 to -1710, were deposited in the GenBank database under the accession numbers shown in Figure 1a. In addition, the complete genomes of representative PEDV field strains were sequenced by the traditional Sanger method. Ten overlapping cDNA fragments spanning the entire genome of each virus strain were RT-PCR-amplified as described previously (Lee & Lee, 2014; Lee et al., 2015, 2017), and each PCR product was sequenced as described above. The 5′ and 3′ ends of the genomes of individual isolates were determined by rapid amplification of cDNA ends (RACE) as described previously (Lee & Lee, 2013). General procedures for DNA manipulation and cloning were performed according to standard procedures (Sambrook & Russell, 2001). The complete genomic sequences of the 2017 viruses were deposited in the GenBank database under the accession numbers shown in Figure 1b.

2.3 | Multiple alignments and phylogenetic analyses

The sequences of the 48 fully sequenced S genes and 31 complete genomes of global PEDV isolates were independently used in sequence alignments and phylogenetic analyses. Multiple sequence alignments were generated with the ClustalX 2.0 program (Thompson, Gibson, Plewniak, Jeanmougin, & Higgins, 1997), and the percentages of nucleotide sequence divergences were further assessed using the same software program. Phylogenetic trees were constructed from the aligned nucleotide or amino acid sequences using the neighbour-joining method and subsequently subjected to bootstrap analysis with 1,000 replicates to determine the percentage reliability values of each internal node of the tree (Saitou & Nei, 1987). All figures involving phylogenetic trees were generated using Mega 4.0 software (Tamura, Dudley, Nei, & Kumar, 2007).

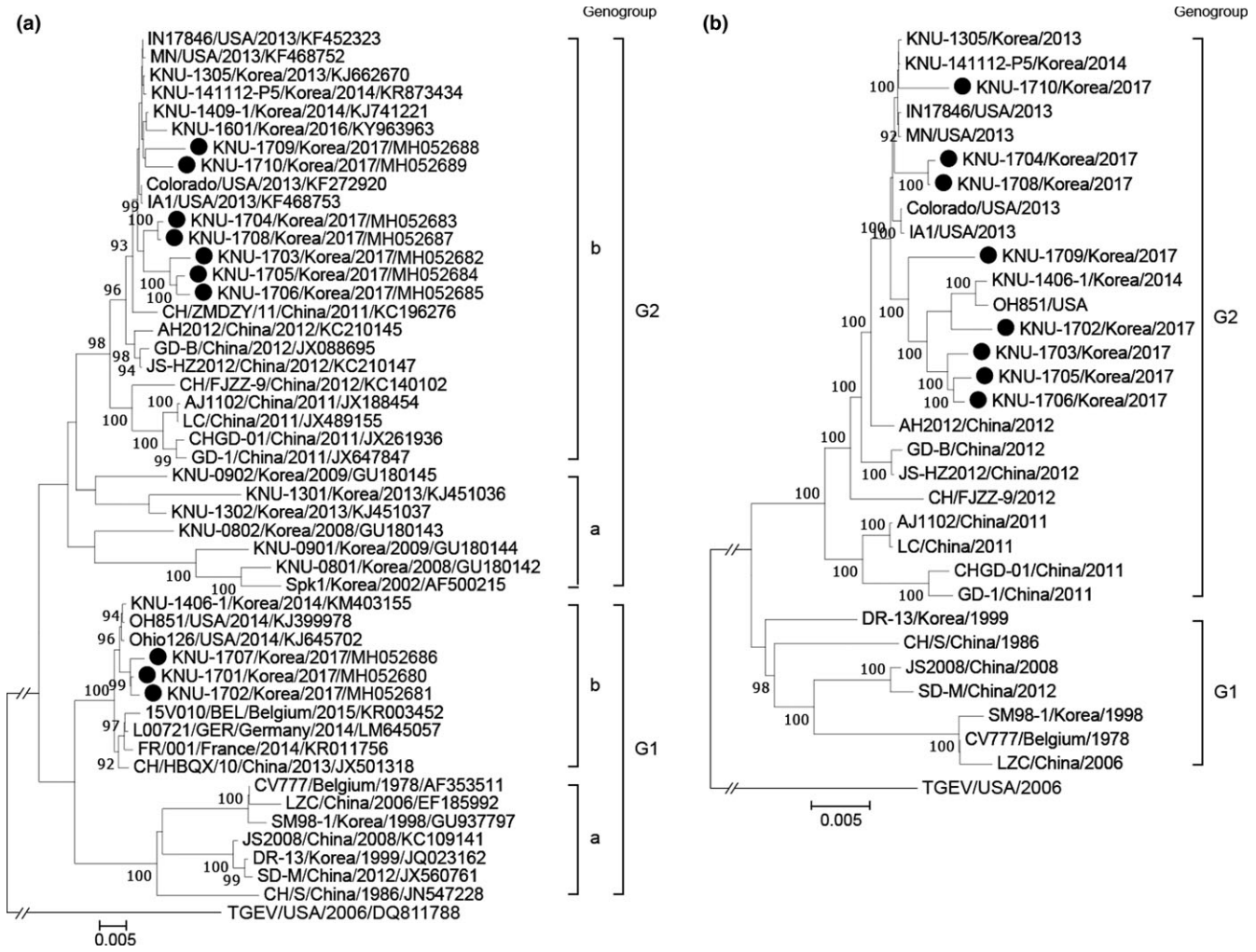

FIGURE 1 Phylogenetic analysis based on nucleotide sequences of the spike genes (a) and full-length genomes (b) of porcine epidemic diarrhoea virus strains. A region of the spike protein and complete sequence of TGEV were included as an outgroup in each tree. Multiple sequence alignments were performed using the ClustalX program, and the phylogenetic tree was constructed from the aligned nucleotide sequences using the neighbour-joining method. Numbers at each branch represent bootstrap values greater than 50% of 1,000 replicates. Names of the strains, countries, years of isolation, GenBank accession numbers, and genogroups and subgroups proposed in this study are shown. The PEDV isolates identified in this study are indicated by solid circles. Scale bars indicate nucleotide substitutions per site

2.4 | Virus isolation and serum neutralization

PEDV isolation was conducted from faecal suspensions on Vero cells in the presence of trypsin (USB, Cleveland, OH) as described previously (Lee et al., 2015). Virus isolation was confirmed by cytopathic effect (CPE) observation, immunofluorescence assay (IFA) and nucleotide sequencing as described previously (Lee et al., 2015). The isolated PEDV strain was propagated for serial passages in Vero cells, and virus titres were determined as described previously (Lee et al., 2015). The cross-reactivity of antisera collected from sows inoculated with a Korean pandemic G2b strain KNU-141112 isolated in 2014 (Baek et al., 2016) was evaluated by a serum neutralization (SN) test in 96-well microtiter plates against the past 2014 and present 2017 isolates as previously described (Lee et al., 2015; Oh, Lee, Choi, & Lee, 2014). The neutralization titre was calculated as the reciprocal of the highest dilution of serum that inhibited virus-specific CPE in all duplicate wells.

3 | RESULTS

3.1 | Genetic and phylogenetic characterizations of 2017 Korean PEDV strains

The PEDV S glycoprotein is a suitable viral gene for investigating genetic relatedness among isolates and the molecular epidemiology of PEDV (Chen et al., 2014; Gerber et al., 2014; Lee, 2015; Lee & Lee, 2014; Lee et al., 2010; Oh et al., 2014). Based on the S gene sequences, therefore, PEDV can be genetically separated into two genogroup clusters, genogroup 1 (G1, classical and recombinant: low-pathogenic) and genogroup 2 (G2, field epizootic or panzootic: high-pathogenic), which are further divided into subgroups 1a and

1b as well as 2a and 2b (Lee, 2015; Lee & Lee, 2014). The complete sequences of the S genes of 10 isolates showed that seven strains were classified as G2b, sharing 98.1%–99.2% amino acid (aa) identity (11–26 aa differences) with the reference Korean G2b strain KNU-141112, whereas the remaining three isolates belonged to G1b, exhibiting 98.7%–99.4% aa homology (8–17 aa differences) with the reference Korean G1b strain KNU-1406 (Table 1; Figure S1). In an interesting manner, the G1b virus S genes were well-conserved, sharing 99.0%–99.7% aa identity with each other, whereas the 2017 G2b S genes were relatively variable, exhibiting 97.1%–99.8% aa homology with each other (Table 1). All 2017 G2b isolates possessed common genetic signatures of genogroup G2, namely S insertions-deletions (S INDELs) comprising two notable 4-aa and 1-aa insertions at positions 55/56 and 135/136, respectively, and a unique 2-aa deletion (DEL) at positions 160 and 161 within the N-terminal hypervariable region of S compared to the prototype CV777 strain (Lee, 2015; Lee & Lee, 2014; Lee et al., 2010) (Figure S2). In a remarkable way, one isolate KNU-1710 was found to contain an additional 2-aa S DEL at positions 137 and 138 in the S1 domain, which was completely absent in the other G1 and G2 isolates in GenBank (Figure S2). Thus, the S gene of KNU-1710 is 4,155 nucleotides (nt) in length encoding a 1384-aa protein, which is 6-nt (2-aa) shorter than the homologous genes in most G2 strains. G1b strains are thought to arise from a recombination event between classical G1a and field epidemic G2 viruses, and their S genes consist of 4,152 nt encoding 1,383 aa residues identical to those of classical G1a strains. They showed typical genetic and phylogenetic features (no S INDELs compared to CV777 and different phylogenetic subgroup [G1b or G2] depending on the sequence of the S protein or whole-genome) (Lee, 2015; Lee, Park, Shin, & Lee, 2014). All G1b isolates identified in this study equally contained the aforementioned S gene characteristics of G1b (Figure S2).

The full-length genomes of one representative G1b and all G2b epidemic strains collected in 2017 were sequenced and analysed to determine their genetic relationships with other global PEDV strains. In particular, a G2b-like KNU-1705 strain contains a unique 39-nt DEL at positions 3,322 and 3,360 located in the ORF1a region potentially encoding nsp3, which acts as a papain-like protease (PL^{pro}). This large ORF1a-DEL pattern has been unprecedented in other G1b and G2b field strains reported in previous and current studies (Figure S3). No additional insertions or deletions were identified in the genomes of all 2017 G1b and G2b isolates. Except for the KNU-1710 and KNU-1705 strains with a notable DEL in S or ORF1a, respectively, the entire genomic sequences of the remaining five 2017 G2b strains were 28,038 nt in length, excluding the 3′ poly(A) tail, while the 2017 G1b KNU-1702 genome is 28,029 nt long as reported previously (Lee & Lee, 2014; Lee, Park et al., 2014). The genome of G1b KNU-1702 shared high nucleotide homology (99.3%) with other complete G1b PEDV genomes in GenBank, showing the lowest nt difference (177-nt) with the Korean G1b strain KNU-1406. Compared to the complete genome of the Korean prototype G2b strain KNU-1305, the genomes of the 2017 G2b strains had 103–255-nt differences (99.0%–99.6% homology) (Table S1). The number of nt/aa differences and percent identity shared between the 2017 isolates and genogroup representative strains is summarized in Table S2.

To establish the genetic relationships involved, phylogenetic analyses were carried out using the nucleotide sequences of the S gene and full-length genome of the 2017 isolates, which were determined in this study and are available from GenBank (Figure 1). Consistent with previous studies (Lee, 2015; Lee & Lee, 2014; Lee et al., 2015), phylogenetic analysis based on the PEDV S genes revealed clear separation among the G1a, G1b, G2a and G2b subgroups. All G2b strains identified in 2017 were grouped within the G2b clade; however, they were in different branches from the emergent US strains and past re-emergent Korean field isolates (Figure 1a). The 2017 G1b isolates were most closely clustered together, forming an independent branch within the G1b subgroup. Furthermore, a phylogenetic tree subsequently reconstructed from the complete genome showed the same grouping structure as the S gene-based tree (Figure 1b). As shown previously (Lee, 2015; Lee et al., 2015), the entire genome-based phylogenetic tree revealed that the G1b strains including KNU-1702 were grouped within the G2 clade because of the similarity between the G1b and G2b genomes, except for the N-terminal one-third of the S gene. These phylogenetic data suggest that the contemporary G1b and G2b epidemic strains detected in 2017 differ from the emergent 2013–2014 pandemic strains in South Korea.

3.2 | Virus isolation and neutralizing activity

We attempted to isolate PEDV from PCR-positive clinical suspensions on Vero cells. One PEDV isolate designated as KNU-1705 was successfully isolated from the faeces of a naturally infected piglet from a commercial farm located in Chungnam Province obtained on December 19, 2017. KNU-1705 produced apparent CPEs typical of PEDV infection, such as cell fusion, syncytium and detachment, in infected Vero cells from passage 3 (P3) (Figure 2a). Virus propagation was confirmed by detecting PEDV antigens by IFA using a PEDV N protein-specific MAb (CAVAC, Daejeon, South Korea). As shown in Figure 2a, distinct staining was distributed in the cytoplasm of typical syncytial cells. In addition, S glycoprotein gene sequencing revealed that the P10 isolate (KNU-180117-P10) contains one nucleotide change, resulting in a nonsynonymous mutation (Ser to Leu) at amino acid position 1,708, compared to the original faecal sample KNU-1705-faeces. Growth kinetics analysis further indicated that KNU-1705 replicated efficiently in Vero cells, reaching a maximum titre of $>10^5$ $TCID_{50}$/ml by 24 hr postinfection (hpi) (Figure 2b). Next, we tested sow antisera previously elicited by a PEDV G2b KNU-141112 strain (Baek et al., 2016; Lee et al., 2015) to determine their cross-neutralization ability against the contemporary KNU-1705 isolate (Figure 2c). In agreement with previous studies, the antisera were greatly effective in inhibiting KNU-141112 infection with mean neutralizing antibody (NA) titres of 1:131. Similar to that, highly diluted antisera efficiently protect Vero cells infected with the recent KNU-1705 isolate with mean NA titres of 1:80,

TABLE 1 Pairwise comparisons of the nucleotide and protein sequences of the S protein genes of the 2017 isolates and genogroup representative porcine epidemic diarrhoea virus strains

Strain name (Genogroup)	CV777	SM98-1	DR-13	OH851	KNU-1406	KNU-1701	KNU-1702	KNU-1707	GD-B	Co/13	KNU-1305	KNU-141112	KNU-1703	KNU-1704	KNU-1705	KNU-1706	KNU-1708	KNU-1709	KNU-1710
CV777 (G1a)		98.8	96.8	95.7	95.7	95.5	95.4	95.3	93.7	93.6	93.6	93.6	93.2	93.4	93.2	93.2	93.4	93.2	93.2
SM98-1 (G1a)	98.3		95.7	94.6	94.6	94.4	94.3	94.2	92.6	92.6	92.5	92.5	92.1	92.3	92.1	92.1	92.4	92.1	92.2
DR-13 (G1a)	96.0	94.5		95.9	95.9	95.7	95.6	95.4	93.6	93.5	93.5	93.5	93.0	93.2	93.1	93.1	93.3	93.0	93.1
OH851 (G1b)	96.1	94.6	96.0		99.9	99.6	99.5	99.4	95.8	96.5	96.3	96.3	95.9	96.3	96.2	96.2	96.4	95.7	95.9
KNU-1406 (G1b)	96.2	94.7	96.0	99.7		99.6	99.5	99.4	95.8	96.4	96.3	96.3	96.0	96.3	96.2	96.2	96.4	95.7	95.9
KNU-1701 (G1b)	96.0	94.5	95.9	99.4	99.4		99.7	99.6	95.6	96.3	96.2	96.1	96.1	96.1	96.4	96.4	96.2	95.5	95.7
KNU-1702 (G1b)	95.7	94.2	95.8	99.2	99.1	99.7		99.5	95.5	96.1	96.0	96.0	96.1	96.0	96.4	96.4	96.1	95.4	95.7
KNU-1707 (G1b)	95.5	94.0	95.5	98.8	98.7	99.3	99.0		95.4	96.1	96.0	95.9	96.0	95.9	96.3	96.3	96.0	95.4	95.6
GD-B (G2b)	93.1	91.6	92.5	95.4	95.3	95.2	94.9	95.0		99.2	99.2	99.2	98.3	98.8	98.4	98.3	98.8	98.4	98.5
Co/13 (G2b)	93.2	91.8	92.8	95.8	95.8	95.6	95.3	95.3	99.4		99.8	99.8	98.9	99.4	99.1	99.0	99.5	99.0	99.1
KNU-1305 (G2b)	93.1	91.6	92.7	95.7	95.6	95.6	95.3	95.2	99.2	99.8		99.9	98.8	99.3	99.0	98.9	99.4	99.1	99.2
KNU-141112 (G2b)	93.0	91.5	92.6	95.6	95.6	95.5	95.2	95.1	99.2	99.7	99.7		98.8	99.3	98.9	98.8	99.4	99.1	99.2
KNU-1703 (G2b)	92.5	91.0	92.0	94.5	94.6	94.9	94.9	94.5	97.9	98.2	98.1	98.1		98.7	99.3	99.2	98.8	98.2	98.3
KNU-1704 (G2b)	93.0	91.5	92.6	95.6	95.6	95.4	95.1	95.1	98.9	99.4	99.2	99.2	97.9		98.8	98.7	99.8	98.7	98.7
KNU-1705 (G2b)	92.5	91.0	92.5	95.2	95.1	95.6	95.6	95.3	98.0	98.5	98.4	98.4	98.4	98.1		99.6	98.9	98.2	98.4
KNU-1706 (G2b)	92.7	91.3	92.6	95.5	95.4	95.8	95.8	95.5	98.1	98.6	98.5	98.4	98.6	98.3	99.4		98.8	98.1	98.3
KNU-1708 (G2b)	93.2	91.7	92.7	95.8	95.7	95.6	95.3	95.1	98.9	99.4	99.2	99.2	97.9	99.7	98.1	98.3		98.7	98.7
KNU-1709 (G2b)	92.4	90.9	91.8	94.6	94.5	94.5	94.2	94.2	98.0	98.5	98.4	98.3	97.1	98.1	97.3	97.4	98.1		98.6
KNU-1710 (G2b)	92.9	91.5	92.3	95.2	95.1	95.0	94.8	94.7	98.2	98.7	98.7	98.6	97.1	98.1	97.4	97.5	98.1	97.6	

The percent nucleotide identity was shown in the upper right and the percent amino acid identity was presented in the lower left.

which were less than 1-$\log_2$ lower but not significantly different compared to those against KNU-141112. Taken together, our data indicate that the antisera cross-reacted well between the homologous G2b field isolates, suggesting antigenic similarity between the 2014 and 2017 PEDV strains.

4 | DISCUSSION

PEDV has emerged or re-emerged as one of the deadliest and most contagious viral pathogens in swine, leading to large financial losses in the global swine industry. Along with strict biosecurity, vaccination is a fundamental tool for managing and eradicating PEDV during epidemic or endemic outbreaks. Although G1a-based vaccines against PEDV were developed and used to combat this disease in South Korea over the past decade, their efficacy in the field, as well as the advantages and disadvantages of their use, is continuously debated. Furthermore, a growing body of evidence suggests that their incomplete effectiveness may result from antigenic, genetic (>10% aa variation between respective S proteins) and phylogenetic (G1 versus G2) differences between vaccine and field epidemic strains (Lee et al., 2010; Lee & Lee, 2014; Oh et al., 2014; Kim et al., 2015; Lee et al., 2015; Lee, 2015). The advent of the 2013–2014 PEDV pandemic led to a breakthrough in the development of G2b-based vaccines phenotypically and genotypically homologous to field strains responsible for global PED epidemics, and these G2b vaccines are currently applied to prevent PEDV in South Korea. Another important policy for controlling PEDV is to operate a monitoring and surveillance system (MOSS) to monitor genetic diversity among field isolates and surveil the emergence of novel variants in the field, which will contribute to preventing future outbreaks. To provide insight into the understanding of the current epidemiological status of PEDV in South Korea, the present study aimed to investigate the genetic, phylogenetic and antigenic characteristics of PEDVs responsible for regional outbreaks in South Korea in 2017.

Nucleotide sequencing analysis revealed that two different PEDV genotypes, low-pathogenic G1b and high-pathogenic G2b, caused regional outbreaks in South Korea, with the latter genotype more prevalent and associated with more serious and fatal clinical outcomes on domestic pig farms. The 2017 isolates exhibited less than

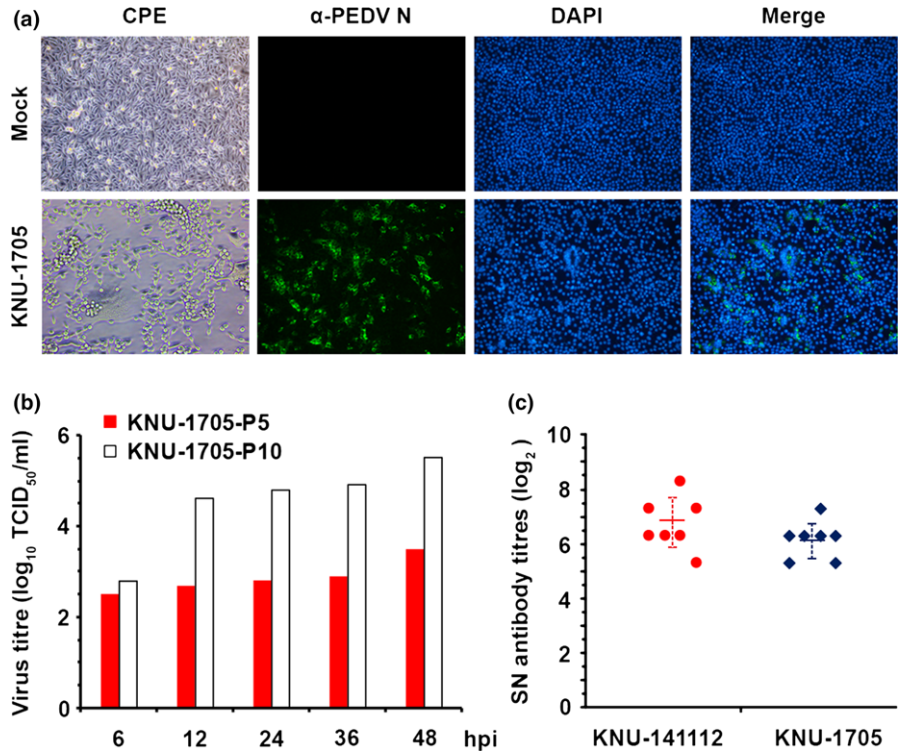

FIGURE 2 Growth properties of KNU-1705. (a) Cytopathology and IFA of porcine epidemic diarrhoea virus isolate KNU-1705 in infected Vero cells. Vero cells were mock-infected or infected with PEDV KNU-1705-P10. PEDV-specific CPEs were observed daily and photographed at 24 hpi using an inverted microscope at a magnification of 200× (first panel). For immunostaining, infected cells were fixed at 24 hpi and incubated with MAb against the N protein, followed by incubation with Alexa green-conjugated goat anti-mouse secondary antibody (second panel). The cells were then counterstained with DAPI (third panels) and examined using a fluorescence microscope at 200× magnification. (b) One-step growth kinetics for KNU-1705 strains. Vero cells were independently infected with PEDV KNU-1705-P5 and -P10. At the indicated time points postinfection, culture supernatants were harvested, and virus titres were determined. Results are expressed as the mean values from triplicate wells, and error bars represent standard derivations. (c) Cross-neutralization between 2014 and 2017 PEDV isolates. Serum samples were subjected to the virus neutralization assays using KNU-141112 (circles) and KNU-1705 (diamonds) strains. Neutralizing antibody titres for individual antisera were spotted as a $\log_2$ scale. Values are representative of the mean from three independent experiments in duplicate and error bars denote standard deviations [Colour figure can be viewed at wileyonlinelibrary.com]

1% nucleotide sequence variations at the genome level with the 2013–2014 pandemic strains. However, field G2b isolates with nearly 2% amino acid sequence divergence compared to previous G2b strains at the S gene level were identified in the present study. Furthermore, mutations within the S protein were randomly and extensively distributed in the S1 and S2 regions among the 2017 isolates (Figure S1). This finding may warn the emergence of new genotypes or variant if broad S mutations are incidentally accumulated in a specific virus in the natural host that is predominant in the field. Moreover, the 2017 G1b and G2b isolates were found to be phylogenetically different from former respective Korean strains, indicating that continuous and independent evolution in the natural host occurs under environmental pressures. In an interesting manner, a large 13-aa DEL in the region of ORF1a encoding nsp3 was identified in G2b KNU-1705 virus, which is the first report of a novel nsp3-DEL variant. Nsp3 critically serves as a PL^{pro} that posttranslationally trims replicase polyproteins into functional nsps during virus replication. However, this unique DEL is in the Glu-rich acidic region, which does not affect the authentic roles of nsp3 and thus is nonessential for coronavirus replication (Lei, Kusov, & Hilgenfeld, 2018). Although the virus can tolerate the large nsp3-DEL which is dispensable for PEDV replication as shown in Figure 2, the pathogenicity of the KNU-1705 virus remains unclear. We have obtained a KNU-1705 isolate that can grow efficiently in cell culture and are currently investigating its biological properties. Based on the high degree of cross-neutralization between the 2014 and 2017 PEDV strains, the antigenicity may be maintained for at least 4 years. This finding indicates the effectiveness of 2013–2014 pandemic strain (KNU-141112)-derived vaccines currently available in South Korea against existing PEDV epidemics. However, consistent with small-scale genetic changes in the 2017 isolates within the identical G2b genotype, antigenic variations appeared to be ongoing under various field conditions. Therefore, the timeline of this situation is unclear and it is unknown whether antigenic differences among PEDV epidemic strains will contribute to the failure of current G2b vaccines. To counteract the prospective scenario, further studies are critical for securing culturable PEDV epidemic strains that are genetically, phenotypically and antigenically characterized in the laboratory.

In summary, genetic and phylogenetic analyses indicated that the 2017 epidemic-related isolates are closely related with corresponding global G1b or G2b strains identified in previous outbreaks and that the virus continues to evolve in its host environment. Despite their genetic diversity, antigenicity currently seems to remain unchanged among G2b strains, indirectly confirming the efficacy of G2b-based vaccines against homologous G2b PEDVs responsible for current epidemics. Because the virus is assumed to undergo an evolutionary process to accumulate mutations to ensure viral fitness in the field, new genotypes or variants of PEDV, against which the current G2b vaccine may provide partial protection, will eventually emerge. Furthermore, this circumstance may advent earlier than expected if PEDV outbreaks fade from our attention following sporadic or endemic outbreaks without serious economic problems. Therefore, it is important to execute mandatory notification of PED-like outbreaks essentially followed by activating an MOSS, including early diagnosis, to survey forthcoming PEDV strains that may emerge locally or globally through genetic drift (e.g., nonsilent point mutations) or genetic shift (e.g., recombination events) and obtain and characterize epidemic field isolates to predict and prepare for future epizootics or panzootics.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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